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FILE 'REGISTRY' ENTERED AT 10:19:50 ON 13 SEP 2006

=> S 3.2.1.20
L1            3 3.2.1.20

=> D 1-3

L1   ANSWER 1 OF 3  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   489295-71-8  REGISTRY
ED   Entered STN:  12 Feb 2003
CN   Glucosidase, α- (Bifidobacterium adolescentis strain DSM 20083 gene
     aglB) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   α-Glucosidase (Bifidobacterium adolescentis strain DSM 20083
     gene aglB)(E.C.3.2.1.20)
CN   GenBank AAL05573
CN   GenBank AAL05573 (Translated from: GenBank AF411186)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   GenBank
LC   STN Files:   CA, CAPLUS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1   ANSWER 2 OF 3  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   341586-71-8  REGISTRY
ED   Entered STN:  15 Jun 2001
CN   Alpha-glucosidase (EC 3.2.1.20) (Lactococcus lactis lactis strain
     IL1403 gene agl) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAK05776
CN   GenBank AAK05776 (Translated from: GenBank AE006398)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1   ANSWER 3 OF 3  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   9001-42-7  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Glucosidase, α- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   α-1,4-Glucosidase
CN   α-D-Glucopyranosidase
CN   α-D-Glucosidase
CN   α-Glucopyranosidase
CN   α-Glucosidase
CN   α-Glucoside hydrolase
CN   E.C. 3.2.1.20
CN   Glucoinvertase
CN   Glucosidoinvertase
CN   Glucosidosucrase
CN   Maltase
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CN   Nitrophenyl α-D-glucosidase
CN   Transglucosidase L
DR   9033-01-6
MF   Unspecified
CI   MAN
LC   STN Files:   ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CABA,
       CAPLUS, CASREACT, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, DDFU,
       DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, NAPRALERT, PIRA, PROMT,
       TOXCENTER, USPAT2, USPATFULL
     Other Sources:   EINECS**, TSCA**
        (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**

            7854 REFERENCES IN FILE CA (1907 TO DATE)
             109 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
            7871 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=> S ALPHA GLUCOSIDASE/CN
L2            0 ALPHA GLUCOSIDASE/CN

=> S ALPHA-GLUCOSIDASE/CN
L3            0 ALPHA-GLUCOSIDASE/CN

=> S A-GLUCOSIDASE/CN
L4            1 A-GLUCOSIDASE/CN

=> D

L4   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2006 ACS on STN
RN   9001-42-7  REGISTRY
ED   Entered STN:  16 Nov 1984
CN   Glucosidase, α- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   α-1,4-Glucosidase
CN   α-D-Glucopyranosidase
CN   α-D-Glucosidase
CN   α-Glucopyranosidase
CN   α-Glucosidase
CN   α-Glucoside hydrolase
CN   E.C. 3.2.1.20
CN   Glucoinvertase
CN   Glucosidoinvertase
CN   Glucosidosucrase
CN   Maltase
CN   Nitrophenyl α-D-glucosidase
CN   Transglucosidase L
DR   9033-01-6
MF   Unspecified
CI   MAN
LC   STN Files:   ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CABA,
       CAPLUS, CASREACT, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, DDFU,
       DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, NAPRALERT, PIRA, PROMT,
       TOXCENTER, USPAT2, USPATFULL
     Other Sources:   EINECS**, TSCA**
        (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**

            7854 REFERENCES IN FILE CA (1907 TO DATE)
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             109 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
            7871 REFERENCES IN FILE CAPLUS (1907 TO DATE)


FILE 'CAPLUS' ENTERED AT 10:22:00 ON 13 SEP 2006

=> S L1;S ALPHA GLUCOSIDASE;S "3.2.1.20"
L5         7872 L1


        1632074 ALPHA
           2485 ALPHAS
        1632180 ALPHA
                  (ALPHA OR ALPHAS)
          17310 GLUCOSIDASE
           2812 GLUCOSIDASES
          18174 GLUCOSIDASE
                  (GLUCOSIDASE OR GLUCOSIDASES)
L6         6170 ALPHA GLUCOSIDASE
                  (ALPHA(W)GLUCOSIDASE)


        6678348 "3"
        8872966 "2"
        8818963 "1"
        2290659 "20"
L7          327 "3.2.1.20"
                  ("3"(W)"2"(W)"1"(W)"20")

=> S CHIMERIC
          49887 CHIMERIC
             31 CHIMERICS
L8        49899 CHIMERIC
                  (CHIMERIC OR CHIMERICS)

=> S SIGNAL PEPTIDE
         487578 SIGNAL
         159276 SIGNALS
         588776 SIGNAL
                  (SIGNAL OR SIGNALS)
         356313 PEPTIDE
         260507 PEPTIDES
         456161 PEPTIDE
                  (PEPTIDE OR PEPTIDES)
L9        15989 SIGNAL PEPTIDE
                  (SIGNAL(W)PEPTIDE)

=> S LYSOSOMAL ENZYME
          24706 LYSOSOMAL
         783687 ENZYME
         454100 ENZYMES
         992316 ENZYME
                  (ENZYME OR ENZYMES)
L10        7790 LYSOSOMAL ENZYME
                  (LYSOSOMAL(W)ENZYME)

=> S L5,L6
L11        9315 (L5 OR L6)

=> S L11(6A)L9
L12           3 L11(6A)L9

=> S L11(10A)L9
L13           6 L11(10A)L9

=> S L11 AND L9
L14          23 L11 AND L9
```

=> S (L12,L13) NOT L14
L15 0 ((L12 OR L13)) NOT L14

=> D L14 1-23 CBIB ABS

L14 ANSWER 1 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
2005:1331045 Document No. 144:65125 Recombinant production of a heterologous secretory protein in a fungal host cell using a signal peptide from cutinase of Humicola insolens. Matsui, Tomoko; Draborg, Henriette; Danielsen, Steffen (Novozymes A/S, Den.). PCT Int. Appl. WO 2005121333 A1 20051222, 48 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-DK387 20050614. PRIORITY: DK 2004-917 20040614.

AB The present invention relates to a method for the recombinant production of a heterologous secretory protein in a fungal host cell such as Aspergillus or Saccharomyces. The method of the invention comprises using a signal peptide from Humicola insolens cutinase, to nucleic acid constructs comprising a first nucleotide sequence encoding the signal peptide and a second nucleotide sequence encoding a polypeptide which is foreign to the first nucleotide sequence. Furthermore, it also relates to expression vectors and host cells comprising said nucleic acid construct. More specifically, the signal peptide coding sequence of the cutinase gene from H. insolens is used for expression of Der p 1 cysteine proteinase from Dermatophagoides pteronyssinus, cellulase from Mucor circinelloides, cysteine protease from Trifolium repens, trypsin from Fusarium, and phytase from Peniophora in Saccharomyces cerevisiae.

L14 ANSWER 2 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
2005:1249587 Document No. 144:229016 Cloning and expression of a gene for an alpha-glucosidase from Saccharomycopsis fibuligera homologous to family GH31 of yeast glucoamylases. Hostinova, Eva; Solovicova, Adriana; Gasperik, Juraj (Institute of Molecular Biology, Slovak Academy of Sciences, Bratislava, 845 51, Slovakia). Applied Microbiology and Biotechnology, 69(1), 51-56 (English) 2005. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer.

AB Cloning of cDNA encoding an α-glucosidase from the dimorphous yeast Saccharomycopsis fibuligera and characterization of the gene product were performed. The cDNA of the putative .alpha .-glucosidase gene consists of 2,886 bp, which includes an open reading frame encoding a 19 amino acid signal peptide at the N-terminal end and a 944 amino acid mature protein with a predicted mol. mass of 105.4 kDa and pI value of 4.52. The deduced amino acid sequence shows a high degree of identity (70%) with two yeast glucoamylases, namely, the extracellular glucoamylase Gam from Schwanniomyces occidentalis and the cell surface glucoamylase Gca from Candida albicans. The recombinant product, synthesized in Saccharomyces cerevisiae, is localized on the cell surface and hydrolyzes maltooligosaccharides exclusively without the ability to digest soluble starch, which is consistent with the specificity characteristic of . alpha.-glucosidase, EC. 3.2.1.20.

L14 ANSWER 3 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
2005:902979 Document No. 143:244080 Therapeutic human acid alpha-glucosidase and it's fragments containing peptide-tags for cation-independent mannose 6 receptor targeting and lysosome internalization. Lebowitz, Jonathan; Maga, John (Zystor Therapeutics, Inc., USA). PCT Int. Appl. WO 2005078077 A2 20050825, 82 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH,

PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US4286 20050210. PRIORITY: US 2004-543812P 20040210.

AB Targeted acid alpha-glucosidase therapeutics that localize to the lysosome are provided. The targeted therapeutics include a therapeutic agent, GAA, and a targeting moiety that binds a receptor on an exterior surface of the cell, permitting proper subcellular localization of the targeted therapeutic upon internalization of the receptor. Nucleic acids, cells, and methods relating to the practice of the invention are also provided. In particular embodiments, IGF-II GILT-tag-containing GAA fusion proteins are prepared according to the following strategies: (1) fusion of the tag at the amino terminus; or (2) insertion of the tag between the trefoil domain and the mature region; or (3) insertion of the tag between the mature region and the C-terminal domain; or (4) fusion of the tag to the C-terminus of a truncated GAA and coexpressing the C-terminal domain. The invention demonstrates that both the trefoil domain and the C-terminal domain are required for the production of functional GAA, and the presence of amino acid residues 792-817 within the C-terminal domain is required for efficient GAA trans-expression. Constructs with various GILT tags (such as 8-28, 41-61, 48-55, 8-87, with R68A mutation), and GAA variants (such as 70-790, 70-952, 880-952, human/mouse GAA hybrid), and/or peptide linkers are tested for increasing the recombinant GAA expression efficiency and proper folding for M6P receptor binding.

L14 ANSWER 4 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2005:60754 Correction of: 2004:1036571 Document No. 142:233342 Correction of: 142:16836 Sequences of human schizophrenia related genes and use for diagnosis, prognosis and therapy. Liew, Choong-Chin (Chondrogene Limited, Can.). U.S. Pat. Appl. Publ. US 2004241727 A1 20041202, 156 pp., Cont.-in-part of U.S. Ser. No. 802,875. (English). CODEN: USXXCO. APPLICATION: US 2004-812731 20040330. PRIORITY: US 1999-115125P 19990106; US 2000-477148 20000104; US 2002-268730 20021009; US 2003-601518 20030620; US 2004-802875 20040312.

AB The present invention is directed to detection and measurement of gene transcripts and their equivalent nucleic acid products in blood. Specifically provided is anal. performed on a drop of blood for detecting, diagnosing and monitoring diseases using gene-specific and/or tissue-specific primers. The present invention also describes methods by which delineation of the sequence and/or quantitation of the expression levels of disease-specific genes allows for an immediate and accurate diagnostic/prognostic test for disease or to assess the effect of a particular treatment regimen. [This abstract record is one of 3 records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

L14 ANSWER 5 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2004:824004 Document No. 141:326804 Thermostable α-glucosidase genes isolated from environmental bacteria and their use for hydrolysis of malto-oligosaccharides and liquefied starch in food processing and dental care products. Gray, Kevin; Garrett, James B.; Aboushadi, Nahla M.; Knowles, Ryan; O'Donoghue, Eileen; Waters, Elizabeth (Diversa Corporation, USA). PCT Int. Appl. WO 2004085615 A2 20041007, 204 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US8541 20040318. PRIORITY: US 2003-456972P 20030320.

AB The invention is directed to polypeptides having a glucosidase activity, including an alpha-glucosidase activity, polynucleotides encoding the polypeptides, and methods for making and using these polynucleotides and polypeptides. Specifically disclosed are protein and cDNA sequences of 12 thermostable (no experiment data) . alpha.-glucosidase isolated from environmental bacteria. In one aspect, the polypeptides of the invention

are used as alpha -glucosidases to catalyze the hydrolysis of starch into sugars, e.g., to convert liquefied starch to glucose. In one aspect, the polypeptides of the invention can catalyze the hydrolysis of both alpha-(1,4) and alpha-(1,6) glucose linkages. In one aspect, the polypeptides of the invention can catalyze the hydrolysis of both malto-oligosaccharides and liquefied starch.

L14 ANSWER 6 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
2004:722964 Document No. 141:230650 Transgenic nonhuman mammals producing in their milk phosphorylated lysosomal proteins with α-glucosidase activity, and compositions and methods for treating enzyme deficiency. Reuser, Arnold J. j.; Van Der Ploeg, Ans T.; Verbeet, Martin P. (Neth.). U.S. Pat. Appl. Publ. US 2004172665 A1 20040902, 58 pp., Cont.-in-part of U.S. Ser. No. 46,180. (English). CODEN: USXXCO. APPLICATION: US 2004-796015 20040310. PRIORITY: US 1995-1796P 19950802; US 1996-700760 19960729; US 2001-770496 20010129; US 2001-14511 20011214; US 2002-46180 20020116.

AB The invention provides transgenic nonhuman mammals producing a lysosomal protein, preferably an enzyme, in their milk, and methods of generating the same. The lysosomal protein is phosphorylated at the 6' position of a mannose side chain residue. Preferably, the lysosomal protein is human acid alpha glucosidase. The invention also provides pharmaceutical compns. for use in enzyme replacement therapy. Also provided are methods of treating Pompe's disease by administrating of produced human α-glucosidase. Animal trial and human clin. trial were conducted using such produced human .alpha .-glucosidase.

L14 ANSWER 7 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
2004:633458 Document No. 141:168974 Transgenes for expression and secretion of human lysosomal acid α-glucosidase from liver and use for gene therapy. Koeberl, Dwight D.; Sun, Baodong (Duke University, USA). PCT Int. Appl. WO 2004064750 A2 20040805, 128 pp. DESIGNATED STATES: W: AE, AE, AG, AL, AL, AM, AM, AM, AT, AT, AU, AZ, AZ, BA, BB, BG, BG, BR, BR, BW, BY, BY, BZ, BZ, CA, CH, CN, CN, CO, CO, CR, CR, CU, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EC, EE, EE, EG, ES, ES, FI, FI, GB, GD, GE, GE, GH, GM, HR, HR, HU, HU, ID, IL, IN, IS, JP, JP, KE, KE, KG, KG, KP, KP, KR, KR, KZ, KZ, KZ, LC, LK, LR, LS, LS, LT, LU, LV, MA, MD, MD, MG, MK, MN, MW, MX, MX, MZ, MZ, NA, NI. (English). CODEN: PIXXD2. APPLICATION: WO 2004-US1453 20040121. PRIORITY: US 2003-441789P 20030122.

AB The invention provides nucleic acids for expressing lysosomal polypeptides such as lysosomal acid α-glucosidase (GAA) and vectors comprising the same. In one embodiment, the invention provides a nucleic acid encoding a chimeric polypeptide comprising a secretory signal sequence operably linked to a lysosomal enzyme. In another embodiment, a nucleic acid sequence is provided comprising a coding region encoding a GAA and a GAA 3' untranslated region (UTR), wherein the GAA 3' UTR comprises a deletion therein. In another embodiment, the invention provides an isolated nucleic acid comprising a coding region encoding a GAA and a 3' UTR, wherein the 3' UTR is less than about 200 nucleotides in length and comprises a segment that is heterologous to the GAA coding region. Also provided are methods of making and using delivery vectors encoding lysosomal GAA, for example, to produce the lysosomal polypeptide or to treat subjects afflicted with a lysosomal storage deficiency. A transgene construct comprising a liver-specific promoter, a synthetic signal peptide sequence, human GAA, and an altered 3'-untranslated region was shown to express and secrete GAA from liver into blood plasma in mice. Furthermore, GAA activity was increased in target tissues of heart, diaphragm, and skeletal muscle. The transgene vector is an AAV (adeno-associated virus) vector and was administered i.v. in immunocompetant, GAA-knockout mice. The results have implications for gene therapy in Pompe disease.

L14 ANSWER 8 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
2003:173758 Document No. 138:237258 Self-processing transgenic plants and plant parts expressing hyperthermophilic processing enzymes. Lanahan, Michael B.; Basu, Shib Sankar; Batie, Christopher J.; Chen, Wen; Craig, Joyce; Kinkema, Mark (Syngenta Participations AG, Switz.). PCT Int. Appl. WO 2003018766 A2 20030306, 158 pp. DESIGNATED STATES: W: AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US27129 20020827. PRIORITY: US 2001-315281P 20010827.

AB The invention provides polynucleotides, preferably synthetic polynucleotides, which encode processing enzymes that are optimized for expression in plants. The polynucleotides encode mesophilic, thermophilic, or hyperthermophilic processing enzymes, which are activated under suitable activating conditions to act upon the desired substrate. Also provided are "self-processing" transgenic plants, and plant parts, e.g., grain, which express one or more of these enzymes and have an altered composition that facilitates plant and grain processing. Methods for making and using these plants, e.g., to produce food products having improved taste and to produce fermentable substrates for the production of ethanol and fermented beverages are also provided.

L14 ANSWER 9 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2002:778103 Document No. 137:274045 Method for isolating manganese peroxidase sequence homologs of Ceriporiopsis subvermispora culture medium using microarrays. Yaver, Debbie; Berka, Randy (Novozymes Biotech, Inc., USA). PCT Int. Appl. WO 2002079400 A2 20021010, 65 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9050 20020312. PRIORITY: US 2001-275283P 20010312.

AB The present invention relates to methods for isolating a gene encoding an enzyme, comprising adding a mixture of labeled first nucleic acid probes from a microbial strain cultured on medium without the substrate, and labeled second nucleic acid probes from a microbial strain cultured on medium with the substrate, to an array of random nucleic acid fragments of the microbial strain. The labeled nucleic acids hybridize to complementary sequences of the genomic fragments in the array, wherein the first nucleic acid probes are labeled with a first reporter and the second nucleic acid probes are labeled with a second reporter. The relative expression of the genes of the microbial strain is determined by the observed hybridization reporter signal of each spot in the array. A gene is isolated from the microbial strain that encodes an enzyme that degrades the substrate. The present invention also relates to isolated genes obtained by such methods.

L14 ANSWER 10 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2001:781078 Document No. 135:348850 Albumin fusion proteins with therapeutic proteins for improved shelf-life. Rosen, Craig A.; Haseltine, William A. (Human Genome Sciences, Inc., USA). PCT Int. Appl. WO 2001079443 A2 20011025, 374 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US11924 20010412. PRIORITY: US 2000-PV229358 20000412; US 2000-PV199384 20000425; US 2000-PV256931 20001221.

AB The present invention encompasses fusion proteins of albumin with various therapeutic proteins. Therapeutic proteins may be stabilized to extend the shelf-life, and/or to retain the therapeutic protein's activity for extended periods of time in solution, in vitro and/or in vivo, by genetically or chemical fusing or conjugating the therapeutic

protein to albumin or a fragment or variant of albumin. Use of albumin fusion proteins may also reduce the need to formulate the protein solns. with large excesses of carrier proteins to prevent loss of therapeutic proteins due to factors such as binding to the container. Nucleic acid mols. encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells. Thus, plasmid vectors are constructed in which DNA encoding the desired therapeutic protein may be inserted for expression of the albumin fusion proteins in yeast (pPPC0005) and mammalian cells (pC4:HSA). Yeast-derived signal sequences from Saccharomyces cerevisiae invertase SUC2 gene, or the stanniocalcin or native human serum albumin signal peptides, are used for secretion in yeast or mammalian systems, resp. Thus, the fusion product of human growth hormone with residues 1-387 of human serum albumin retains essentially intact biol. activity after 5 wk of incubation in tissue culture media at 37°, whereas recombinant human growth hormone used as control lost its biol. activity in the first week. Although the potency of the albumin fusion proteins is slightly lower than the unfused counterparts in rapid bioassays, their biol. stability results in much higher biol. activity in the longer term in vitro assay or in vivo assays. Addnl., the present invention encompasses pharmaceutical compns. comprising albumin fusion proteins and methods of treating, preventing, or ameliorating diseases, disorders or conditions using albumin fusion proteins of the invention.

L14 ANSWER 11 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2001:634531 Document No. 136:258038 Analysis of the chromosome sequence of the legume symbiont Sinorhizobium meliloti strain 1021. Capela, Delphine; Barloy-Hubler, Frederique; Gouzy, Jerome; Bothe, Gordana; Ampe, Frederic; Batut, Jacques; Boistard, Pierre; Becker, Anke; Boutry, Marc; Cadieu, Edouard; Dreano, Stephane; Gloux, Stephanie; Godrie, Therese; Goffeau, Andre; Kahn, Daniel; Kiss, Erno; Lelaure, Valerie; Masuy, David; Pohl, Thomas; Portetelle, Daniel; Puhler, Alfred; Purnelle, Benedicte; Ramsperger, Ulf; Renard, Clotilde; Thebault, Patricia; Vandenbol, Micheline; Weidner, Stefan; Galibert, Francis (Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, Unite Mixte de Recherche (UMR) 215 Centre National de la Recherche Scientifique (CNRS), Institut National de la Recherche Agronomique, Chemin, Tolosan, F-31326, Fr.). Proceedings of the National Academy of Sciences of the United States of America, 98(17), 9877-9882 (English) 2001. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB Sinorhizobium meliloti is an α-proteobacterium that forms agronomically important N2-fixing root nodules in legumes. We report here the complete sequence of the largest constituent of its genome, a 62.7% GC-rich 3654,135-bp circular chromosome. Annotation allowed assignment of a function to 59% of the 3341 predicted protein-coding ORFs, the rest exhibiting partial, weak, or no similarity with any known sequence. Unexpectedly, the level of reiteration within this replicon is low, with only two genes duplicated with more than 90% nucleotide sequence identity, transposon elements accounting for 2.2% of the sequence, and a few hundred short repeated palindromic motifs (RIME1, RIME2, and C) widespread over the chromosome. Three regions with a significantly lower GC content are most likely of external origin. Detailed annotation revealed that this replicon contains all housekeeping genes except two essential genes that are located on pSymB. Amino acid/peptide transport and degradation and sugar metabolism appear as two major features of the S. meliloti chromosome. The presence in this replicon of a large number of nucleotide cyclases with a peculiar structure, as well as of genes homologous to virulence determinants of animal and plant pathogens, opens perspectives in the study of this bacterium both as a free-living soil microorganism and as a plant symbiont.

L14 ANSWER 12 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2001:558549 Document No. 135:367972 The receptor of Bacillus sphaericus binary toxin in Culex pipiens (Diptera: Culicidae) midgut: molecular cloning and expression. Darboux, I.; Nielsen-LeRoux, C.; Charles, J.-F.; Pauron, D. (Unite Sante Vegetale et Environnement, Institut National de la Recherche Agronomique, Antibes, 06606, Fr.). Insect Biochemistry and Molecular Biology, 31(10), 981-990 (English) 2001. CODEN: IBMBES. ISSN: 0965-1748. Publisher: Elsevier Science Ltd..

AB Culex pipiens larval midgut is the primary target of the binary toxin (Bin) present in parasporal inclusions of Bacillus sphaericus. Cpm1, a 60-kDa protein purified from brush border membranes, has been proposed as the receptor of the Bin toxin in the midgut epithelial cells of mosquitoes. We have cloned and characterized the corresponding cDNA from midgut of Culex pipiens larvae. The open reading frame predicted a 580 amino-acid protein with a putative signal peptide at the N-terminus and a putative GPI-anchoring signal at the C-terminus. The amino acid sequence of the cloned Cpm1 exhibited 39-43% identities with insect maltases (α-glucosidases and α-amylases). Recombinant Cpm1 expressed in E. coli specifically bound to the Bin toxin and had a significant α- glucosidase activity but no α-amylase activity. These results support the view that Cpm1 is an α- glucosidase expressed in Culex midgut where it constitutes the receptor for the Bin toxin. To date, this is the first component involved in the mosquitocidal activity of the Bacillus sphaericus Bin toxin to be characterized. Its identification provides a key step to elucidate the mode of action of the Bin toxin and the mechanisms of resistance developed against it by some mosquito strains.

L14 ANSWER 13 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2001:465529 Document No. 136:113567 Cloning and expression pattern of a gene encoding an α-xylosidase active against xyloglucan oligosaccharides from Arabidopsis. Sampedro, Javier; Sieiro, Carmen; Revilla, Gloria; Gonzalez-Villa, Tomas; Zarra, Ignacio (Departamento de Biologia Vegetal, Laboratorio de Fisiologia Vegetal, Facultad de Biologia, Universidad de Santiago de Compostela, Santiago de Compostela, E-15782, Spain). Plant Physiology, 126(2), 910-920 (English) 2001. CODEN: PLPHAY. ISSN: 0032-0889. Publisher: American Society of Plant Physiologists.

AB An α-xylosidase active against xyloglucan oligosaccharides was purified from cabbage (Brassica oleracea var. capitata) leaves. Two peptide sequences were obtained from this protein, the N-terminal and an internal one, and these were used to identify an Arabidopsis gene coding for an α-xylosidase that we propose to call AtXYL1. It has been mapped to a region of chromosome I between markers at 100.44 and 107.48 cM. AtXYL1 comprised three exons and encoded a peptide that was 915 amino acids long, with a potential signal peptide of 22 amino acids and eight possible N-glycosylation sites. The protein encoded by AtXYL1 showed the signature regions of family 31 glycosyl hydrolases, which comprises not only α-xylosidases, but also α- glucosidases. The α-xylosidase activity is present in apoplastic extns. from Arabidopsis seedlings, as suggested by the deduced signal peptide. The first eight leaves from Arabidopsis plants were harvested to analyze α-xylosidase activity and AtXYL1 expression levels. Both increased from older to younger leaves, where xyloglucan turnover is expected to be higher. When this gene was introduced in a suitable expression vector and used to transform Saccharomyces cerevisiae, significantly higher α-xylosidase activity was detected in the yeast cells. α-Glucosidase activity was also increased in the transformed cells, although to a lesser extent. These results show that AtXYL1 encodes for an apoplastic α-xylosidase active against xyloglucan oligosaccharides that probably also has activity against p-nitrophenyl-α-D-glucoside.

L14 ANSWER 14 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

2000:818937 Document No. 134:146439 Expression and Characterization of Glycosylated and Catalytically Active Recombinant Human α-Galactosidase A Produced in Pichia pastoris. Chen, Yingsi; Jin, Ming; Egborge, Tobore; Coppola, George; Andre, Jamie; Calhoun, David H. (Department of Chemistry, City College of New York, New York, NY, 10031, USA). Protein Expression and Purification, 20, 472-484 (English) 2000. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic Press.

AB Fabry disease is an X-linked inborn error of glycolipid metabolism caused by deficiency of the lysosomal enzyme α-galactosidase A. This enzyme is responsible for the hydrolysis of terminal α-galactoside linkages in various glycolipids. An improved method of production of recombinant α-galactosidase A for use in humans is needed in order to develop new approaches for enzyme therapy. Human α-galactosidase A for use in enzyme therapy has previously been obtained from human sources and from recombinant clones derived from human cells, CHO cells, and insect cells. In this report we describe the construction of clones of the methylotrophic yeast Pichia pastoris that

produce recombinant human α-galactosidase A. Recombinant human α-galactosidase A is secreted by these Pichia clones and the level of production is more than 30-fold greater than that of previously used methods. Production was optimized using variations in temperature, pH, cDNA copy number, and other variables using shake flasks and a bioreactor. Expression of the human enzyme increased with increasing cDNA copy number at 25°C, but not at the standard growth temperature of 30°C. The recombinant α-galactosidase A was purified to homogeneity using ion exchange (POROS 20 CM, POROS 20 HQ) and hydrophobic (Toso-ether, Toso-butyl) chromatog. with a BioCAD HPLC Workstation. Purified recombinant α-galactosidase A was taken up by fibroblasts derived from Fabry disease patients and normal enzyme levels could be restored under these conditions. Anal. of the carbohydrate present on the recombinant enzyme indicated the predominant presence of N-linked high-mannose structures rather than complex carbohydrates. (c) 2000 Academic Press.

L14 ANSWER 15 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

1999:753358 Document No. 132:9622 Aspergillus DDC2 and DDC3 genes sequences and their use for enhanced protein production in mutant cells of filamentous fungi. Wahleithner, Jill; Christensen, Tove (Novo Nordisk Biotech, Inc., USA; Novo Nordisk A/s). PCT Int. Appl. WO 9960136 A1 19991125, 78 pp. DESIGNATED STATES: W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US10689 19990514. PRIORITY: US 1998-79601 19980515; US 1998-79344 19980515.

AB The present invention relates to methods for enhanced protein production, comprising (a) cultivating a mutant of a parent filamentous fungal cell in a nutrient medium suitable for production of the protein, wherein (i) the mutant cell comprises a first nucleic acid sequence encoding the protein of interest and a modification of one or more of second nucleic acid sequences encoding DDC2 and/or DDC3 proteins, and (ii) the mutant cell produces more of the protein than the parent cell when cultured under the same conditions; and (b) recovering the protein from the nutrient medium of the mutant cell. The present invention also relates to the DDC2 and DDC3 proteins cDNA and genomic sequences, and nucleic acid constructs, recombinant expression vectors, and host cells comprising the sequences. The present invention further relates to mutants of filamentous fungal cells and methods for obtaining the mutant cells.

L14 ANSWER 16 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

1999:234011 Document No. 130:263131 Production of proteins in plant seeds using seed maturation-specific promoters and signal sequences. Lemaux, Peggy G.; Cho, Myeong-je; Buchanan, Robert B. (The Regents of the University of California, USA). PCT Int. Appl. WO 9916890 A2 19990408, 48 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US20691 19980930. PRIORITY: US 1997-60510 19970930.

AB Methods for producing proteins in plant seeds are disclosed. Expression of the protein is driven by a seed-specific promoter and the protein is preferably expressed as a fusion polypeptide that includes a signal peptide that causes the protein to accumulate in a subcellular compartment to protect the protein. The functionality of the barley B1-hordein and D-hordein promoters were tested with and without the 19 amino acid N-terminal signal peptide sequence using both transient and stable expression assays in barley. The B1-hordein promoter with the signal sequence exhibited the same expression pattern, but with stronger expression. PCR anal. confirms the present of reporter uidA and bar genes in genomic DNA from T0 plants of different stably transformed lines. Also disclosed are improved methods for transforming plants, including cereals such as barley, corn or wheat.

L14 ANSWER 17 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

1997:532524 Document No. 127:145926 Potato α-glucosidase cDNA sequence, antisense sequences, recombinant enzyme, and genetic engineering plants with modified starch breakdown. Taylor, Mark Andrew; Davies, Howard Vivian (Nickerson Biocem Limited, UK; Taylor, Mark Andrew; Davies, Howard Vivian). PCT Int. Appl. WO 9724448 A1 19970710, 39 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1996-GB3239 19961224. PRIORITY: GB 1995-26613 19951228.

AB The present invention provides recombinant or isolated nucleic acid encoding α-glucosidase, especially those nucleic acid sequences encoding a plant α-glucosidase. Antisense nucleic acid is also provided, as well as the use of both the isolated or recombinant sequences and the antisense sequences. Uses of the invention include enhancing and reducing expression of .alpha .-glucosidases and the provision of novel starches. Examples include the potato cultivar Record gene MAL1 α- glucosidase cDNA sequence and recombinant production Plants transformed with vectors containing the MAL1 sequence in antisense orientation had altered starch turnover rates and maltose and sucrose contents.

L14 ANSWER 18 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

1996:517973 Document No. 125:215543 Molecular cloning of a gene encoding acid α-glucosidase from Tetrahymena pyriformis. Alam, Md. Shariful; Nakashima, Shigeru; Deyashiki, Yoshihiro; Banno, Yoshiko; Hara, Akira; Nozawa, Yoshinori (School Medicine, Gifu University, Gifu, 500, Japan). Journal of Eukaryotic Microbiology, 43(4), 295-303 (English) 1996. CODEN: JEMIED. ISSN: 1066-5234. Publisher: Society of Protozoologists.

AB Lysosomal acid α-glucosidase is essential for the degradation of glycogen to glucose in lysosomes. The ciliated protozoan Tetrahymena pyriformis secretes acid α-glucosidase into its culture medium. We have earlier reported the purification and characterization of acid α-glucosidase from T. pyriformis. The exact mol. mechanism of secretion of this enzyme has not yet been clarified. In the present study we have isolated a full length cDNA clone encoding acid α-glucosidase from T. pyriformis. The isolated clone (3019 bp) contained an open reading encoding 923 amino acids, and has an estimated mol. mass of 104 kDa. Northern blot anal. revealed that the isolated cDNA hybridized to a 2.8-kb mRNA transcript. N-terminal amino acids after the first methionine fulfilled the requirement of a signal peptide. The deduced amino acid sequence contains the amino acid sequences determined of several peptides derived from the purified enzyme, and was found to have 34% identity and 45% similarity with that of human lysosomal enzyme, with 75% identity in the 16 amino acids at the proposed active site.

L14 ANSWER 19 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN

1996:100647 Document No. 124:198312 Two genes encoding midgut-specific maltase-like polypeptides from Anopheles gambiae. Zheng, Liangbiao; Whang, Lucy H.-S.; Kumar, Vasantha; Kafatos, Fotis C. (Biological Laboratories, Harvard University, Cambridge, MA, 02138, USA). Experimental Parasitology, 81(3), 272-83 (English) 1995. CODEN: EXPAAA. ISSN: 0014-4894. Publisher: Academic.

AB Full-length cDNA clones of two genes have been isolated from the African malaria vector mosquito, Anopheles gambiae. These genes, designated Agm1 and Agm2, encode maltase-like polypeptides of 498 and 599 residues, resp. Deduced amino acid sequences contain a putative signal peptide sequence and four potential glycosylation sites. Agm1 and Agm2 show highest similarities to the Mal1 gene from Aedes aegypti and three clustered maltase genes from Drosophila melanogaster. Both genes are located at position 46D, in the terminal division of the left arm of the third chromosome. Agm2 has very strict tissue and temporal specificity, being expressed exclusively in the adult midgut. The

specificity of Agm1 is similar but appears slightly broader; transcripts of this gene are detected at a low level in the pupae, and occasionally in the adult carcass after removal of the midgut.

L14 ANSWER 20 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
1994:215765 Document No. 120:215765 Transgenic potatoes with suppression of tuber sprouting. Von Schaewen, Antje; Sonnewald, Uwe; Willmitzer, Lothar (Institut fuer Genbiologische Forschung Berlin Gmbh, Germany). Ger. Offen. DE 4213444 A1 19931028, 7 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1992-4213444 19920418.

AB Transgenic potato plants are prepared in which sprouting of the tubers during storage is suppressed by lowering the sucrose concentration through a decrease in the activity of starch-degrading enzymes and/or an increase in sucrose degradation The plants are transformed e.g. with DNA containing an invertase gene in the sense orientation and a gene for amylase, starch phosphorylase, maltase, maltose phosphorylase, UDPG pyrophosphorylase, sucrose phosphate synthase, or sucrose phosphate phosphatase in the antisense orientation. Thus, potato plants were transformed with an Agrobacterium tumefaciens vector containing plasmid p35S-CW-INV. This plasmid contained a constitutive promoter from cauliflower mosaic virus, a portion of a potato proteinase inhibitor II gene fused to the invertase (suc2) gene from yeast and a signal peptide sequence, and a polyadenylation signal. The regenerated potato plants showed a >100-fold increase in acid invertase activity, a 20-fold decrease in sucrose concentration, and a 20-fold increase in glucose and fructose content in the apoplastic space.

L14 ANSWER 21 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
1993:119860 Document No. 118:119860 Structural and functional changes of lysosomal acid α-glucosidase during intracellular transport and maturation. Wisselaar, Heleen A.; Kroos, Marian A.; Hermans, Monique M. P.; Van Beeumen, Jos; Reuser, Arnold J. J. (Dep. Cell Biol. Genet., Erasmus Univ., Rotterdam, 3000, Neth.). Journal of Biological Chemistry, 268(3), 2223-31 (English) 1993. CODEN: JBCHA3. ISSN: 0021-9258.

AB The synthesis and posttranslational modification of lysosomal acid . alpha.-glucosidase were studied in a cell-free translation system and in mammalian cells transfected with acid . alpha.-glucosidase cDNA constructs. The newly synthesized precursor, sequestered in the endoplasmic reticulum, was demonstrated to be membrane-bound by lack of signal peptide cleavage, and to be catalytically inactive. Sugar chain modification was shown to occur in the Golgi complex and to be dependent on the rate of transport. From the trans-Golgi network different routes were found to be followed by acid α-glucosidase. A fraction of precursor mols., proteolytically released from the membrane anchor, appeared to enter the secretory pathway and was recovered from the cell culture medium in a catalytically active form. A second fraction was transported to the lysosomes and was trimmed in a stepwise process at both the amino- and carboxyl-terminal ends. The intramol. cleavage sites were determined Involvement of thiol proteinases was demonstrated. Specificity for the natural substrate glycogen was gained during the maturation process. The phosphomannosyl receptor is assumed to be instrumental in the lysosomal targeting of acid α-glucosidase, but a phosphomannosyl receptor-independent transport of membrane-bound precursor mols. to the lysosomes, either directly or via the plasma membrane, cannot be excluded.

L14 ANSWER 22 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
1993:35039 Document No. 118:35039 Primary structure and processing of the Candida tsukubaensis α-glucosidase. Homology with the rabbit intestinal sucrase-isomaltase complex and human lysosomal α-glucosidase. Kinsella, B. Therese; Hogan, Stephane; Larkin, Angela; Cantwell, Barbara A. (Guinness Brew. Worldwide Res. Cent., Dublin, Ire.). European Journal of Biochemistry, 202(2), 657-64 (English) 1991. CODEN: EJBCAI. ISSN: 0014-2956.

AB The nucleotide sequence of a 4.39-kb DNA fragment encoding the . alpha.-glucosidase gene of C. tsukubaensis is reported. The cloned gene contains a major open reading frame (ORF 1) which encodes the α-glucosidase as a single precursor polypeptide of 1070 amino acids with a predicted mol. mass of 119 kDa. N-terminal amino acid sequence anal. of the individual subunits of the purified enzyme, expressed in the recombinant host

Saccharomyces cerevisiae, confirmed that the α-glucosidase precursor is proteolytically processed by removal of an N-terminal signal peptide to yield the two peptide subunits 1 and 2, of mol. masses 63-65 kDa and 50-53 kDa, resp. Both subunits are secreted by the heterologous host S. cerevisiae in a glycosylated form. Coincident with its efficient expression in the heterologous host, the C. tsukubaensis α-glucosidase gene contains many of the canonical features of highly expressed S. cerevisiae genes. There is considerable sequence similarity between C. tsukubaensis .alpha .-glucosidase, the rabbit sucrase-isomaltase complex (proSI) and human lysosomal acid α-glucosidase. The cloned DNA fragment from C. tsukubaensis contains a second open reading frame (ORF 2) which has the capacity to encode a polypeptide of 170 amino acids. The function and identity of the polypeptide encoded by ORF 2 is not known.

L14 ANSWER 23 OF 23 CAPLUS COPYRIGHT 2006 ACS on STN
1989:34574 Document No. 110:34574 Primary structure and processing of lysosomal α-glucosidase; homology with the intestinal sucrase-isomaltase complex. Hoefsloot, Lies H.; Hoogeveen-Westerveld, Marianne; Kroos, Marian A.; Van Beeumen, Jos; Reuser, Arnold J. J.; Oostra, Ben A. (Dep. Cell Biol. Genet., Erasmus Univ., Rotterdam, 3000 DR, Neth.). EMBO Journal, 7(6), 1697-704 (English) 1988. CODEN: EMJODG. ISSN: 0261-4189.

AB Lysosomal α-glucosidase (acid maltase) is essential for degradation of glycogen in lysosomes. Enzyme deficiency results in glycogenosis type II. The amino acid sequence of the entire enzyme was derived from the nucleotide sequence of cloned cDNA. The cDNA comprises 3636 nucleotides, and hybridizes with a mRNA of .apprx.3.6 kb, which is absent in fibroblasts of 2 patients with glycogenosis type II. The encoded protein has a mol. mass of 104.645 kd and starts with a signal peptide. Sites of proteolytic processing are established by identification of N-terminal amino acid sequences of the 110-kd precursor, and the 76-fold and 70-kd mature forms of the enzyme encoded by the cDNA. Interestingly, both N-terminal and C-terminal processing occurs. Sites of sugar-chain attachment are proposed. A remarkable homol. is observed between this soluble lysosomal α- glucosidase and the membrane-bound intestinal brush border sucrase-isomaltase enzyme complex. It is proposed that these enzymes are derived from the same ancestral gene. Around the putative active site of sucrase and isomaltase, 10 out of 13 amino acids are identical to the corresponding amino acids of lysosomal α-glucosidase. This strongly suggests that the aspartic acid residue at this position is essential for catalytic function of lysosomal α-glucosidase.

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=> E KOEBERL D/AU
=> S E3-E7
             3 "KOEBERL D"/AU
             1 "KOEBERL D C"/AU
            11 "KOEBERL D D"/AU
             1 "KOEBERL DWIGHT"/AU
            20 "KOEBERL DWIGHT D"/AU
L16         36 ("KOEBERL D"/AU OR "KOEBERL D C"/AU OR "KOEBERL D D"/AU OR "KOEB
               ERL DWIGHT"/AU OR "KOEBERL DWIGHT D"/AU)

=> E BAODONG S/AU
=> E BAODONG/AU

=> S L16 AND L11
L17          7 L16 AND L11

=> S L17 NOT L14
L18          6 L17 NOT L14

=> D 1-6 CBIB ABS
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L18 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN
2005:1165660 Document No. 144:21708 Evasion of Immune Responses to Introduced Human Acid α-Glucosidase by Liver-Restricted Expression in Glycogen Storage Disease Type II. Franco, Luis M.; Sun, Baodong; Yang, Xiaoyi; Bird, Andrew; Zhang, Haoyue; Schneider, Ayn; Brown, Talmage; Young, Sarah P.; Clay, Timothy M.;

Amalfitano, Andrea; Chen, Y. T.; Koeberl, Dwight D. (Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC, 27710, USA). Molecular Therapy, 12(5), 876-884 (English) 2005. CODEN: MTOHCK. ISSN: 1525-0016. Publisher: Elsevier.

AB Glycogen storage disease type II (GSD-II; Pompe disease) is caused by a deficiency of acid α-glucosidase (GAA; acid maltase) and manifests as muscle weakness, hypertrophic cardiomyopathy, and respiratory failure. Adeno-associated virus vectors containing either a liver-specific promoter (LSP) (AAV-LSPhGAApA) or a hybrid CB promoter (AAV-CBhGAApA) to drive human GAA expression were pseudotyped as AAV8 and administered to immunocompetent GAA-knockout mice. Secreted hGAA was detectable in plasma between 1 day and 12 wk post-administration with AAV-LSPhGAApA and only from 1 to 8 days post-administration for AAV-CBGAApA. No anti-GAA antibodies were detected in response to AAV-LSPhGAApA (<1:200), whereas AAV-CBhGAApA provoked an escalating antibody response starting 2 wk post-administration. The LSP drove approx. 60-fold higher GAA expression than the CB promoter in the liver by 12 wk following vector administration. Furthermore, the detected cellular immunity was provoked by AAV-CBhGAApA, as detected by ELISpot and CD4+/CD8+ lymphocyte immunodetection. GAA activity was increased to higher than normal and glycogen content was reduced to essentially normal levels in the heart and skeletal muscle following administration of AAV-LSPhGAApA. Thus, liver-restricted GAA expression with an AAV vector evaded immunity and enhanced efficacy in GSD-II mice.

L18 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2005:462026 Document No. 143:186627 Correction of glycogen storage disease type II by an adeno-associated virus vector containing a muscle-specific promoter. Sun, Baodong; Zhang, Haoyue; Franco, Luis M.; Brown, Talmage; Bird, Andrew; Schneider, Ayn; Koeberl, Dwight D. (Department of Pediatrics, Division of Medical Genetics, Duke University Medical Center, Durham, NC, 27710, USA). Molecular Therapy, 11(6), 889-898 (English) 2005. CODEN: MTOHCK. ISSN: 1525-0016. Publisher: Elsevier.

AB Glycogen storage disease type II (Pompe disease) causes death in infancy from cardiorespiratory failure due to acid α- glucosidase (GAA; acid maltase) deficiency. An AAV2 vector pseudotyped as AAV6 (AAV2/6 vector) transiently expressed high-level human GAA in GAA-knockout (GAA-KO) mice without reducing glycogen storage; however, in immunodeficient GAA-KO/SCID mice the AAV2/6 vector expressed high-level GAA and reduced the glycogen content of the injected muscle for 24 wk. A CD4+/CD8+ lymphocytic infiltrate was observed in response to the AAV2/6 vector in immunocompetent GAA-KO mice. When a muscle-specific creatine kinase promoter was substituted for the CB promoter (AAV-MCKhGAApA), that AAV2/6 vector expressed high-level GAA and reduced glycogen content in immunocompetent GAA-KO mice. Muscle-restricted expression of hGAA provoked only a humoral (not cellular) immune response. I.v. administration of a high number of particles of AAV-MCKhGAApA as AAV2/7 reduced the glycogen content of the heart and skeletal muscle and corrected individual myofibers in immunocompetent GAA-KO mice 24 wk postinjection. In summary, persistent correction of muscle glycogen content was achieved with an AAV vector containing a muscle-specific promoter in GAA-KO mice, and this approach should be considered for muscle-targeted gene therapy in Pompe disease.

L18 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2004:1046141 Document No. 142:148356 Efficacy of an adeno-associated virus 8-pseudotyped vector in glycogen storage disease type II. Sun, Baodong; Zhang, Haoyue; Franco, Luis M.; Young, Sarah P.; Schneider, Ayn; Bird, Andrew; Amalfitano, Andrea; Chen, Y.-T.; Koeberl, Dwight D. (Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC, 27710, USA). Molecular Therapy, 11(1), 57-65 (English) 2005. CODEN: MTOHCK. ISSN: 1525-0016. Publisher: Elsevier.

AB Glycogen storage disease type II (GSD-II; Pompe disease) causes death in infancy from cardiorespiratory failure. The underlying deficiency of acid α-glucosidase (GAA; acid maltase) can be corrected by liver-targeted gene therapy in GSD-II, if secretion of GAA is accompanied by receptor-mediated uptake in cardiac and skeletal muscle. An adeno-associated virus (AAV) vector encoding human (h) GAA was pseudotyped as AAV8 (AAV2/8) and injected i.v. into immunodeficient GSD-II mice. High levels of hGAA were maintained in plasma for 24 wk following AAV2/8 vector administration. A marked increase in vector copy number in the liver was demonstrated for the AAV2/8 vector

compared to the analogous AAV2/2 vector. GAA deficiency in the heart and skeletal muscle was corrected with the AAV2/8 vector in male GSD-II mice, consistent with receptor-mediated uptake of hGAA. Male GSD-II mice demonstrated complete correction of glycogen storage in heart and diaphragm with the AAV2/8 vector, while female GSD-II mice had correction only in the heart. A biomarker for GSD-II was reduced in both sexes following AAV2/8 vector administration. Therefore, GAA production with an AAV2/8 vector in a depot organ, the liver, generated evidence for efficacious gene therapy in a mouse model for GSD-II.

L18 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2003:892544 Document No. 139:354442 Methods for producing recombinant adeno-associated virus vectors and use for gene therapy. Amalfitano, Andrea; Koeberl, Dwight D.; Sun, Baodong (Duke University, USA).
PCT Int. Appl. WO 2003092594 A2 20031113, 85 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US13323 20030430. PRIORITY: US 2002-376397P 20020430.

AB The present invention provides methods for producing recombinant adeno-associated virus (AAV) vectors using a novel hybrid adenovirus comprising a recombinant AAV vector genome embedded within the adenovirus backbone and their uses as gene therapy vectors. Specifically, the recombinant hybrid virus includes (a) a deleted adenovirus vector genome comprising the adenovirus 5' and 3' cis-elements for viral replication and encapsidation, and further comprising a deletion in an adenovirus genomic region selected from the group consisting of: (i) the polymerase region, wherein said deletion prevents the expression of a functional polymerase protein, (ii) the preterminal protein region, wherein said deletion prevents the expression of a functional preterminal protein, and (iii) both the regions of (i) and (ii); and (b) a recombinant AAV vector genome flanked by the adenovirus vector genome sequences of (a), said recombinant AAV vector genome comprising (i) AAV 5' and 3' inverted terminal repeats, (ii) an AAV packaging sequence, and (iii) a heterologous nucleic acid sequence, wherein said heterologous nucleic acid sequence is flanked by the 5' and the 3' AAV inverted terminal repeats of (i).

L18 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN

2003:327137 Document No. 139:31447 Packaging of an AAV vector encoding human acid α-glucosidase for gene therapy in glycogen storage disease type II with a modified hybrid adenovirus-AAV vector. Sun, Baodong; Chen, Y.-T.; Bird, Andrew; Xu, Fang; Hou, Yang-Xun; Amalfitano, Andrea; Koeberl, Dwight D. (Division of Medical Genetics, Duke University Medical Center, Durham, NC, 27710, USA). Molecular Therapy, 7(4), 467-477 (English) 2003. CODEN: MTOHCK. ISSN: 1525-0016. Publisher: Elsevier Science.

AB The authors have developed an improved method for packaging adeno-associated virus (AAV) vectors with a replication-defective adenovirus-AAV (Ad-AAV) hybrid virus. The AAV vector encoding human acid α- glucosidase (hGAA) was cloned into an E1, polymerase/preterminal protein-deleted adenovirus, such that it is packaged as an Ad vector. Importantly, the Ad-AAV hybrid cannot replicate during AAV vector packaging in 293 cells, because of deletion of polymerase/preterminal protein. The residual Ad-AAV in the AAV vector stock was reduced to <1 infectious particle per 1010 AAV vector particles. These modifications resulted in .apprx.30-fold increased packaging of the AAV vector for the hybrid Ad-AAV vector method as compared with standard transfection-only methods. Similarly improved packaging was demonstrated for pseudotyping the AAV vector as AAV6, and for AAV vector packaging with a second Ad-AAV vector encoding canine glucose-6-phosphatase. Liver-targeted delivery of either the Ad-AAV hybrid or AAV vector particles in acid .alpha .-glucosidase-knockout (GAA-KO) mice revealed secretion of hGAA with the Ad-AAV vector, and sustained secretion of hGAA with an AAV vector in hGAA-tolerant GAA-KO mice. Further development of hybrid Ad-AAV vectors could offer distinct advantages for gene therapy in glycogen storage diseases.

L18 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2006 ACS on STN
2003:125088 Document No. 139:95182 Long-term correction of glycogen storage disease type II with a hybrid Ad-AAV vector. Sun, Bao-dong; Chen, Y.-T.; Bird, Andrew; Amalfitano, Andrea; Koeberl, Dwight D. (Division of Medical Genetics, Duke University Medical Center, Durham, NC, 27710, USA). Molecular Therapy, 7(2), 193-201 (English) 2003. CODEN: MTOHCK. ISSN: 1525-0016. Publisher: Elsevier Science.

AB We administered an adenovirus-adeno-associated virus (Ad-AAV) vector encoding human acid α-glucosidase (hGAA) to acid . alpha.-glucosidase-knockout (GAA-KO) mice on day 3 of life by gastrocnemius injection. In contrast to previous results for muscle-targeted Ad vector in adult GAA-KO mice, the muscles of the hindlimb showed reduced glycogen content and persistent hGAA for as long as 6 mo after neonatal Ad-AAV vector administration. Not only the injected gastrocnemius muscles, but also the hamstrings and quadriceps muscles produced therapeutic levels of hGAA as a result of widespread transduction with the Ad-AAV vector; moreover, hGAA activity was 50-fold elevated as compared to normal mice. Vector RNA was detected in the hindlimb muscles, the hearts, and the livers by northern blot anal. and/or by RT-PCR for as long as 6 mo. The low levels of hGAA detected in the heart were attributable to transduction with the Ad-AAV vector, not to secretion of hGAA by the injected muscle and uptake by the heart. Finally, although an antibody response to hGAA was present, it did not prevent the correction of glycogen storage in the skeletal muscle of GAA-KO mice.

=> S L16 AND L9
L19 1 L16 AND L9

=> S L19 NOT (L18,L14)
L20 0 L19 NOT ((L18 OR L14))

	L #	Hits	Search Text	DBs
1	L1	53653	CHIMERIC	US-PGPUB; USPAT
2	L2	55941	SIGNAL ADJ (PEPTIDE OR SEQUENCE)	US-PGPUB; USPAT
3	L3	3883	ALPHA ADJ GLUCOSIDASE	US-PGPUB; USPAT
4	L4	30	L3 SAME L1	US-PGPUB; USPAT
5	L5	25	L4 AND L2	US-PGPUB; USPAT
6	L6	5	L4 NOT L5	US-PGPUB; USPAT
7	L7	178266	SECRET$	US-PGPUB; USPAT
8	L8	2546	L7 AND L3	US-PGPUB; USPAT
9	L9	740	L8 AND L1	US-PGPUB; USPAT
10	L10	771	L7 SAME L3	US-PGPUB; USPAT
11	L11	125	L10 AND L1	US-PGPUB; USPAT
12	L12	771	L7 SAME L3	US-PGPUB; USPAT
13	L13	125	L12 AND L1	US-PGPUB; USPAT
14	L14	1	L12 SAME L1	US-PGPUB; USPAT
15	L15	1199	L7 NEAR6 L1	US-PGPUB; USPAT
16	L16	301	L7 NEAR6 L3	US-PGPUB; USPAT
17	L17	55	L16 AND L1	US-PGPUB; USPAT

18	L18	0	L16 SAME L1	US-PGPUB; USPAT

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FILE 'CAPLUS' ENTERED AT 11:20:48 ON 13 SEP 2006

=> S PICHIA;S METHANOL;S SIGNAL(W)(SIGNAL OR PEPTIDE)
L1          7315 PICHIA


          198187 METHANOL
             696 METHANOLS
L2        198553 METHANOL
                 (METHANOL OR METHANOLS)


          487578 SIGNAL
          159276 SIGNALS
          588776 SIGNAL
                 (SIGNAL OR SIGNALS)
          487578 SIGNAL
          159276 SIGNALS
          588776 SIGNAL
                 (SIGNAL OR SIGNALS)
          356313 PEPTIDE
          260507 PEPTIDES
          456161 PEPTIDE
                 (PEPTIDE OR PEPTIDES)
L3         16142 SIGNAL(W)(SIGNAL OR PEPTIDE)

=> S L1 AND L2
L4          1080 L1 AND L2

=> S PROMOTER
          177184 PROMOTER
           57872 PROMOTERS
L5        200104 PROMOTER
                 (PROMOTER OR PROMOTERS)

=> S L2 (6A) L5
L6           559 L2 (6A) L5

=> S L6 AND L1
L7           147 L6 AND L1

=> S L7 AND L3
L8            19 L7 AND L3

=> D 1-19 CBIB ABS

L8   ANSWER 1 OF 19  CAPLUS  COPYRIGHT 2006 ACS on STN
2006:62718  Identification and recombination expression of a bacterial
     exolevanase useful for the production of high fructose syrups.  Menendez,
     Carmen; Hernandez, Lazaro; Pais, Jose M.; Banguela, Alexander; Ramirez,
     Ricardo; Trujillo, Luis E.; Alfonso, Dubiel; Arrieta, Juan G. (Plant
     Division, Centre for Genetic Engineering and Biotechnology, Havana, Cuba).
     Biotecnologia Aplicada, 22(1), 68-72 (English) 2005.  CODEN: BTAPEP.
     ISSN: 0864-4551.  Publisher: Elfos Scientiae.
AB   The sugarcane endophyte Gluconacetobacter diazotrophicus produces levan from sucrose by
     a secreted levansucrase (LsdA).  A levanase-encoding gene (IsdB) was identified
     starting 52 bp downstream of the IsdA gene. Recombinant expression of the IsdB gene in
     Escherichia coli and Pichia pastoris resulted in a functional protein capable of
     hydrolyzing levan and inulin to free fructose without the formation of oligofructans,
     indicating exo-type activity.  LsdB was efficiently secreted into the P. pastoris
     culture medium driven by the Saccharomyces cerevisiae alpha-factor signal peptide using
     either the methanol-inducible AOX1 or the constitutive GAP promoter.  The recombinant
     protein was not glycosylated at its single potential N-glycosylation site.  The GAP
     promoter-driven expression of the IsdB gene did not cause cell toxicity and provided
     for a three-fold higher productivity (26.6 U mL-1; 39 h fermentation) than the
     methanol-inducible system (21.1 U mL-1; 96 h fermentation).  We conclude that the P.
     pastoris constitutive system provides a convenient alternative for the large-scale
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production and secretion of LsdB, an enzyme com. attractive to convert polyfructans into high fructose syrups.

L8 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1291992 Document No. 144:32200 Ricin-like toxin precursors cleavable by disease-specific proteinases for treatment of cancer, viral or parasitic infections. Borgford, Thor; Braun, Curtis; Purac, Admir; Stoll, Dominik (Can.). U.S. Pat. Appl. Publ. US 2005272048 A1 20051208, 495 pp., Cont.-in-part of U.S. 89,058. (English). CODEN: USXXCO. APPLICATION: US 2004-893584 20040719. PRIORITY: US 1997-45148P 19970430; US 1997-63715P 19971029; WO 1998-CA394 19980430; US 1999-157807P 19991004; US 1999-403752 19991029; US 2000-551151 20000414; US 2000-197409P 20000414; WO 2000-CA1162 20001004; US 2002-89058 20020919.

AB Ricin precursors with the ricin A and B chains linked by a protease-labile linker peptide are described for use in the treatment of disease. The linker peptide contains a cleavage site for a disease specific protease such as a cancer, viral or parasitic protease. The ricin A or B chains may be replaced by comparable cytotoxic proteins such as the abrin A chain. The protein is delivered to the target tissue using viral vectors carrying an expression cassette for the ricin fusion protein gene. Construction of a series of variants of preproricin cleavable by a number of different proteinases and their recombinant expression in yeast is described.

L8 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1154578 Document No. 143:417264 Production of insulinotropic peptides with transgenic yeast. Melarkode, Ramakrishnan; Sriram, Akundi Venkata; Sastry, Kedarnath Nanjund; Suryanarayan, Shrikumar (Biocon Limited, India). PCT Int. Appl. WO 2005100388 A1 20051027, 41 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-IN118 20050418. PRIORITY: IN 2004-CH352 20040419.

AB The insulinotropic peptide, exendin-4, is overexpressed in Pichia pastoris using a codon optimized encoding sequence with a optional signal peptide and/or propeptide. The expression of the exogenous gene is driven by the methanol inducible promoter AOX. The purified peptide from the recombinant organism is alpha amidated at the penultimate serine. The production of exendin-4 may also be carried out in bacteria such as E. coli or in yeast such as Saccharomyces cerevisiae. The purified recombinant peptide is useful in formulating pharmaceutical compns. for the treatment of diseases such as diabetes and obesity.

L8 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2004:936355 Document No. 142:369641 Expression of recombinant Chinese bovine enterokinase catalytic subunit in P. pastoris and its purification and characterization. Fang, Lei; Sun, Qi-Ming; Hua, Zi-Chun (The State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, Peop. Rep. China). Acta Biochimica et Biophysica Sinica, 36(7), 513-517 (English) 2004. CODEN: ABBSC2. ISSN: 1672-9145. Publisher: Shanghai Scientific and Technical Publishers.

AB Enterokinase is a tool protease widely utilized in the cleavage of recombinant fusion proteins. CDNA encoding the catalytic subunit of Chinese bovine enterokinase (EKL) was amplified by PCR and then fused to the 3' end of prepro secretion signal peptide gene of α-mating factor from Saccharomyces cerevisiae to get the α-MF signal-EKL-His6 encoding gene by PCR. Then the whole coding sequence was cloned into the integrative plasmid pAO815 under the control of a methanol-inducible promoter and transformed GS115 methylotrophic strain of Pichia pastoris. Secreted expression of recombinant EKL-His6 was attained by methanol induction and its mol. weight is 43 kDa. Because of the existence of His6-tag, EKL-His6 was easily purified from P. pastoris fermentation supernatant by using Ni2+ affinity chromatog. and the yield is 5.4 mg per L of

fermentation culture. This purified EKL-His6 demonstrates excellent cleavage activity towards fusion protein containing EK cleavage site.

L8 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
2004:771014 Document No. 141:407782 Cloning and characterization of gluconolactone oxidase of Penicillium cyaneo-fulvum ATCC 10431 and evaluation of its use for production of D-erythorbic acid in recombinant Pichia pastoris. Salusjaervi, Tuomas; Kalkkinen, Nisse; Miasnikov, Andrei N. (Danisco Innovation, Kantvik, Finland). Applied and Environmental Microbiology, 70(9), 5503-5510 (English) 2004. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.
AB A D-erythorbic acid-forming soluble flavoprotein, gluconolactone oxidase (GLO), was purified from Penicillium cyaneo-fulvum strain ATCC 10431 and partially sequenced. Peptide sequences were used to isolate a cDNA clone encoding the enzyme. The cloned gene (accession number AY576053) exhibits high levels of similarity with the genes encoding other known eukaryotic lactone oxidases and also with the genes encoding some putative prokaryotic lactone oxidases. Anal. of the coding sequence of the GLO gene indicated the presence of a typical secretion signal sequence at the N terminus of GLO. No other targeting or anchoring signals were found, suggesting that GLO is the first known lactone oxidase that is secreted rather than targeted to the membranes of the endoplasmic reticulum or mitochondria. Exptl. evidence, including the N-terminal sequence of mature GLO and data on glycosylation and localization of the enzyme in native and recombinant hosts, supports this anal. The GLO gene was expressed in Pichia pastoris, and recombinant GLO was produced by using the strong methanol-induced AOX1 promoter. In order to evaluate the suitability of purified GLO for production of D-erythorbic acid, we immobilized it on N-hydroxysuccinimide-activated Sepharose and found that the immobilized GLO retained full activity during immobilization but was rather unstable under reaction conditions. Our results show that both soluble and immobilized forms of GLO can, in principle, be used for production of D-erythorbic acid from D-glucono-δ-lactone or (in combination with glucose oxidase and catalase) from glucose. We also demonstrated the feasibility of glucose-D-erythorbic acid fermentation with recombinant strains coexpressing GLO and glucose oxidase genes, and we analyzed problems associated with construction of efficient D-erythorbic acid-producing hosts.

L8 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
2004:577355 Document No. 141:308532 Expression of the human extracellular domain of high density lipoprotein receptor in methylotropic yeast. Hu, Jian; Hong, Bin; Li, Yuan (Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences / Peking Union Medical College, Beijing, 100050, Peop. Rep. China). Yichuan Xuebao, 30(1), 20-24 (Chinese) 2003. CODEN: ICHPCG. ISSN: 0379-4172. Publisher: Kexue Chubanshe.
AB The expression of the human extracellular domain of high d. lipoprotein receptor (HDLR) in the methylotropic yeast Pichia pastoris is reported. The DNA fragment encoding extracellular domain of HDLR was cloned by RT-PCR from Hepatoma Bel-7402 total RNA, and the cloned cDNA was verified by sequencing. The cDNA was subcloned into pPIC9K, and the integrative secretory expression plasmid of Pichia pastoris was constructed with the methanol-inducible alc. oxidase promoter and α-signal peptide. The recombinant plasmid was transformed into Pichia pastoris GS1 15 (His- strain) by electroporation. PCR was used to confirm the insertion of HDLR gene into the genome of Mut+ transformants. The recombinant strain was induced by 1% CH3OH for cultivation, and the supernatant was analyzed by SDS-PAGE and Western blot. The result shows a specific protein band at approx. 64.5 kDa after 5 days of induction. The binding to acetylated LDL (AcLDL) is confirmed using the DiI-AcLDL as ligand.

L8 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
2004:484877 Document No. 141:224051 High-level expression of Candida parapsilosis lipase/acyltransferase in Pichia pastoris. Brunel, Laetitia; Neugnot, Virginie; Landucci, Laure; Boze, Helene; Moulin, Guy; Bigey, Frederic; Dubreucq, Eric (UMR Ingenierie des reactions biologiques, bioproductions (IR2B), Equipe de Genie Microbiologique et Enzymatique, Universite Montpellier II, Montpellier, F-34060, Fr.). Journal of Biotechnology, 111(1), 41-50 (English) 2004. CODEN: JBITD4. ISSN:

0168-1656. Publisher: Elsevier Science B.V..

AB Candida parapsilosis has been previously shown to produce a lipase/acyltransferase (EC 3.1.1.3) that preferentially catalyzes transfer reactions such as alcoholysis over hydrolysis in the presence of suitable nucleophiles other than water, even in aqueous media (aw>0.9). This enzyme has been shown to belong to a new family of lipases. The present work describes the cloning of the gene coding for this lipase/acyltransferase in the yeast Pichia pastoris and the heterologous high-level expression of the recombinant enzyme. The lipase/acyltransferase gene, in which the sequence encoding the signal peptide was replaced by that of the α-factor of Saccharomyces cerevisiae, was placed under the control of the methanol inducible promoter of the alc. oxidase 1 gene (AOX1). A transformed P. pastoris clone, containing five copies of the lipase/acyltransferase gene, was selected for the production of recombinant enzyme. The fed-batch culture supernatant contained 5.8 g l-1 (weighted) of almost pure recombinant lipase/acyltransferase displaying the same catalytic behavior as the original enzyme.

L8 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2004:252592 Document No. 140:265627 Expression of human insulin using yeast protein expression secretion system. Sahib, Maharaj K.; Raju, Edupuganti B.; Shaligram, Umesh S. (Wockhardt Limited, India). PCT Int. Appl. WO 2004024862 A2 20040325, 24 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-IB3773 20030908. PRIORITY: US 2002-2002/PV410774 20020913.

AB This invention discloses expression of prepro-insulin polypeptides. The DNA construct for expression of prepro-insulin has formula: pY - SP - B(1-29)-A(1-21), where pY is any promoter in yeast and SP encodes a signal peptide region that enables the secretion of polypeptides expressed in yeasts and lies to the N-terminus of the insulin peptide region B(1-29)-A(1-21). B(1-29)-A(1-21) encodes, upon expression, the insulin peptide region in which B(1-29) is the B chain of insulin from amino acid 1 to amino acid 29, A(1-21) is the A chain of insulin from amino acid 1 to amino acid 21, and that the amino acid 29 of the B chain directly connects, by means of a peptide bond, the amino acid 1 of the A chain. The N-terminal region directs the polypeptides efficiently into the secretory pathway of yeasts. Modifications at the N-terminal region, just adjacent to the insulin polypeptide region, further increase the efficiency of secretion and improves the final yield of secreted insulin. Thus a combination of such promoters and precursors with the said N-terminal regions appear to function as very high yielding expression systems in yeasts. The invention further relates to purification of prepro-insulin and insulin-t-Bu ester-t-Bu ether by cation exchange chromatog., isoelec. precipitation, and reverse phase HPLC.

L8 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2004:243489 Document No. 141:22262 Functional production and secretion of the Gluconacetobacter diazotrophicus fructose-releasing exo-levanase (LsdB) in Pichia pastoris. Menendez, Carmen; Hernandez, Lazaro; Banguela, Alexander; Pais, Jose (Plant-Microbe Interactions Laboratory, Plant Division, Center for Genetic Engineering and Biotechnology (CIGB), Havana, 10600, Cuba). Enzyme and Microbial Technology, 34(5), 446-452 (English) 2004. CODEN: EMTED2. ISSN: 0141-0229. Publisher: Elsevier Science.

AB The gene encoding the fructose-releasing exo-levanase (LsdB; EC 3.2.1.65) from Gluconacetobacter diazotrophicus SRT4 was expressed in Pichia pastoris using either the methanol-inducible AOX1 or the constitutive GAP promoter. In both systems, the recombinant LsdB was efficiently secreted into the culture medium driven by the Saccharomyces cerevisiae alpha-factor signal peptide. The levanase activity reached 21.1 U ml-1 in the culture supernatant of methanol-induced cells grown for 96 h to a final d. of 115.2 g l-1 (dry weight) under fed-batch conditions. The GAP promoter-driven expression of the lsdB gene did not cause cell toxicity and provided for a

higher LsdB yield (26.6 U ml-1; 0.46 g l-1) despite the fermentation time was only 39 h and the biomass reached 59.7 g l-1 (dry weight). The constitutively produced LsdB containing a C-terminal His6-tag fusion was purified to homogeneity from the culture supernatant by nickel affinity chromatog. with a process recovery of 77.3%. The purified enzyme, which was not glycosylated at its single potential N-glycosylation site, showed a maximal specific activity (58 U mg-1) for the substrate levan at pH 5.0 and 30 °C. The enzyme also hydrolyzed inulin, raffinose, and sucrose, but not melezitose. The reaction on levan and inulin resulted in the successive release of free fructose without the formation of intermediate oligofructans. We conclude that the P. pastoris GAP promoter based system provides a convenient alternative for the large-scale production and secretion of LsdB, an enzyme com. attractive to convert polyfructans into high fructose syrups.

L8 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2004:8980 Document No. 140:286218 Secretory production of Zymomonas mobilis levansucrase by the methylotrophic yeast Hansenula polymorpha. Park, Buem-Seek; Vladimir, Ananin; Kim, Chul Ho; Rhee, Sang-Ki; Kang, Hyun Ah (Metabolic Engineering Laboratory, Korea Research Institute of Bioscience and Biotechnology, Daejon, 305-333, S. Korea). Enzyme and Microbial Technology, 34(2), 132-138 (English) 2004. CODEN: EMTED2. ISSN: 0141-0229. Publisher: Elsevier Science.

AB To direct the secretory expression of Zymomonas mobilis levansucrase (LevU) in the methylotrophic yeast Hansenula polymorpha, the Z. mobilis levU gene was fused to the inulinase signal sequence under the control of the methanol oxidase promoter (PMOX). The vector containing the PMOX-levU expression cassette was introduced into H. polymorpha and multiply integrated into the host chromosomes. Recombinant LevU was expressed as an active and soluble form in H. polymorpha transformants, but the recombinant protein accumulated mainly inside the cells, representing about 5% of total cell proteins in shake-flask cultivations. Interestingly, a significant fraction of the recombinant LevU was secreted into the culture supernatant with a maximum yield of 12,200 U/l using fed-batch fermns. with methanol feeding. H. polymorpha-derived recombinant LevU displayed catalytic activities comparable to those of the Escherichia coli-derived recombinant LevU, producing levans with high mol. wts.

L8 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2003:125137 Document No. 139:2721 Methylotrophic yeast Pichia pastoris as a host for production of ATP-diphosphohydrolase (apyrase) from potato tubers (Solanum tuberosum). Nourizad, Nader; Ehn, Maria; Gharizadeh, Baback; Hober, Sophia; Nyren, Pal (AlbaNova University Center, Department of Biotechnology, SCFAB, Royal Institute of Technology, Stockholm, SE-106 91, Swed.). Protein Expression and Purification, 27(2), 229-237 (English) 2003. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB ATP-diphosphohydrolase (apyrase) catalyzes the hydrolysis of phosphoanhydride bonds of nucleoside tri- and di-phosphates in the presence of divalent cations. This enzyme has broad substrate specificity for nucleotides, which makes it an ideal enzyme for different biotech. applications, such as DNA sequencing and platelet-aggregation inhibition. The only com. available apyrase is isolated from potato tubers. To avoid batch-to-batch variations in activity and quality, we decided to produce a recombinant enzyme. The methylotrophic yeast Pichia pastoris was chosen as an eukaryotic expression host. The coding sequence of potato apyrase, without the signal peptide, was cloned into the YpDC541 vector to create a fusion with the α-mating secretion signal of Saccharomyces cerevisiae. The gene was placed under the control of the methanol-inducible alc. oxidase promoter. The YpDC541-apyrase construct was integrated into P. pastoris strain SMD1168. Methanol induction resulted in secretion of apyrase to a level of 1 mg/L. The biol. active recombinant apyrase was purified by hydrophobic interaction and ion exchange chromatog. According to SDS-PAGE and Western blot anal., the purified enzyme was shown to be hyperglycosylated. By enzymic removal of N-glycans, a single band corresponding to a mol. mass of 48 kDa was detected. The recombinant apyrase was found to function well when used in combination with Pyrosequencing technol.

L8 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2003:97522 Document No. 138:148681 Expression systems using methylotrophic yeast hosts for manufacture of proteins for therapeutic use. Clark, Mike A. (Phoenix Pharmacologics, Inc., USA). PCT Int. Appl. WO 2003010288 A2 20030206, 67 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US23586 20020725. PRIORITY: US 2001-915815 20010726.

AB Expression vectors for use in methylotrophic yeasts are described. Methylotrophic yeasts as expression hosts allow the rapid and cheap fermentation of proteins using simple media without the need to remove pyrogens. The expression vectors use the promoter of the methanol -inducible AOX gene to bring high-level, tightly-regulated expression of the transgene. Construction of vectors for the manufacture of human tumor necrosis factor is described. Intracellular manufacture of the protein was without the formation of inclusion bodies. The purified protein had an IC50 of 52 pg/mL against L929 cells compared to a value of 133 pg/mL for the protein from Escherichia coli. Secretory expression using the signal peptide of the Saccharomyces cerevisiae α-mating factor is also demonstrated.

L8 ANSWER 13 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2001:673171 Document No. 136:36046 Equine herpesvirus 1 glycoprotein D expressed in Pichia pastoris is hyperglycosylated and elicits a protective immune response in the mouse model of EHV-1 disease. Ruitenberg, K. M.; Gilkerson, J. R.; Wellington, J. E.; Love, D. N.; Whalley, J. M. (Division of Environmental and Life Sciences, Department of Biological Sciences, Macquarie University, Sydney, 2109, Australia). Virus Research, 79(1-2), 125-135 (English) 2001. CODEN: VIREDF. ISSN: 0168-1702. Publisher: Elsevier Science Ireland Ltd..

AB Equine herpesvirus 1 glycoprotein D (EHV-1 gD) has been shown in mouse models and in the natural host to have potential as a subunit vaccine, using various expression systems that included Escherichia coli, baculovirus and plasmid DNA. With the aim of producing secreted recombinant protein, the authors have cloned and expressed EHV-1 gD, lacking its native signal sequence and C-terminal transmembrane region, into the methylotrophic yeast Pichia pastoris. The truncated glycoprotein D (gD) gene was placed under the control of the methanol inducible alc. oxidase 1 promoter and directed for secretion with the Saccharomyces cerevisiae α-factor prepro secretion signal. SDS-PAGE and Western blot anal. of culture supernatant fluid 24 h after induction revealed gD-specific protein products between 40 and 200 kDa. After treatment with PNGase F and Endo H, three predominant bands of 34, 45 and 48 kDa were detected, confirming high mannose N-linked glycosylation of Pichia-expressed gD (Pic-gD). N-terminal sequence anal. of PNGase F-treated affinity-purified protein showed that the native signal cleavage site of gD was being recognized by P. pastoris and the 34 kDa band could be explained by internal proteolytic cleavage effected by a putative Kex2-like protease. Pic-gD, when used in a DNA prime/protein boost inoculation schedule, induced high EHV-1 ELISA and virus neutralizing antibodies and provided protection from challenge infection in BALB/c mice.

L8 ANSWER 14 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

2001:192708 Document No. 134:217991 Recombinant human serum albumin, its expression vectors and manufacture in Pichia pastoris. Liu, Zhimin (Maoji Biological Engineering Science and Tech. Co., Ltd., Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1266100 A 20000913, 85 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 1999-102794 19990304.

AB The invention discloses recombinant human serum albumin (rHSA), its expression vectors and manufacture in Pichia(P.) pastoris. The rHSA chimeric gene has a methanol-inducible promoter from alc. oxidase 1 (AOX1) gene, Kozak sequence (AXXATG), HSA coding region linked to the coding sequence for prepro-signal peptide from Saccharomyces cerevisiae AMF (alpha-mating factor), and 3' transcription terminator (including polyA signal) from AOX1 gene. The signal peptide will be cleaved by yeast KEX-2 proteinase

(at Lys-Arg) upon its secretion in P. pastoris. The invention provides two expression vectors (pHSA201 and pHSA301) and a series of transformed P. pastoris strains (either Mut- or Mut+ phenotype) including GS115:pHSA201S1 to GS115:pHSA201S4 and GS115:pHSA301S1 to GS115:pHSA301S3. The invention also provides fermentation method for manufacturing recombinant HSA in these genetic engineered P. pastoris and the yield is up to 4-6g/L.

L8 ANSWER 15 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
2001:192707 Document No. 134:217990 Recombinant human serum albumin, its expression vectors and manufacture in Pichia pastoris. Liu, Zhimin (Maoji Biological Engineering Science and Technology Co., Ltd., Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1266099 A 20000913, 85 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 1999-102745 19990304.

AB The invention discloses recombinant human serum albumin (rHSA), its expression vectors and manufacture in Pichia(P.) pastoris. The rHSA chimeric gene has a methanol-inducible promoter from alc. oxidase 1 (AOX1) gene, Kozak sequence (AXXATG), HSA coding region linked to the coding sequence for prepro-signal peptide from Saccharomyces cerevisiae AMF (alpha-mating factor), and 3' transcription terminator (including polyA signal) from AOX1 gene. The signal peptide will be cleaved by yeast KEX-2 proteinase (at Lys-Arg) upon its secretion in P. pastoris. The invention provides two expression vectors (pHSA201 and pHSA301) and a series of transformed P. pastoris strains (either Mut- or Mut+ phenotype) including GS115:pHSA201S1 to GS115:pHSA201S4 and GS115:pHSA301S1 to GS115:pHSA301S3. The invention also provides fermentation method for manufacturing recombinant HSA in these genetic engineered P. pastoris and the yield is up to 4-6g/L.

L8 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
1997:641852 Document No. 127:327122 High-level production of recombinant Geotrichum candidum lipases in yeast Pichia pastoris. Holmquist, Mats; Tessier, Daniel C.; Cygler, Miroslaw (Natl. Res. Council of Canada, Biotechnol. Res. Inst., Montreal, QC, H4P 2R2, Can.). Protein Expression and Purification, 11(1), 35-40 (English) 1997. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic.

AB We describe the heterologous high-level expression of the two Geotrichum candidum lipase (GCL) isoenzymes from strain ATCC 34614 in the methylotrophic yeast Pichia pastoris. The lipase cDNAs were placed under the control of the methanol-inducible alc. oxidase promoter. The lipases expressed in P. pastoris were fused to the α-factor secretion signal peptide of Saccharomyces cerevisiae and were secreted into the culture medium. Cultures of P. pastoris expressing lipase accumulated active recombinant enzyme in the supernatant to levels of .apprx.60 mg/L virtually free from contaminating proteins. This yield exceeds that previously reported with S. cerevisiae by a factor of more than 60. Recombinant GCL I and GCL II had mol. masses of .apprx.63 and .apprx.66 kDa, resp., as determined by SDS-PAGE. The result of endoglucosidase H digestion followed by Western blot anal. of the lipases suggested that the enzymes expressed in P. pastoris received N-linked high-mannose-type glycosylation to an extent, 6-8% (weight/weight), similar to that in G. candidum. The specific activities and substrate specificities of both recombinant lipases were determined and were found to agree with what has been reported for the enzymes isolated from the native source.

L8 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
1997:8912 Document No. 126:27681 Cloning and expression of human bile salt-stimulated lipase cDNA. Das, Goutam (Astra Aktiebolag, Swed.). Can. Pat. Appl. CA 2172447 AA 19960924, 38 pp. (English). CODEN: CPXXEB. APPLICATION: CA 1996-2172447 19960322. PRIORITY: IN 1995-MA351 19950323; SE 1995-1939 19950524.

AB The invention relates to DNA mols., recombinant vectors and cell cultures for use in methods for expression of bile salt-stimulated lipase (BSSL) in the methylotrophic yeast Pichia pastoris.

L8 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN
1996:252868 Document No. 124:308857 Expression and secretion of rabbit

plasma cholesteryl ester transfer protein by Pichia pastoris. Kotake, Hidetoshi; Li, Qianqian; Ohnishi, Taira; Ko, Kerry W. S.; Agellon, Luis B.; Yokoyama, Shinji (Lipid Lipoprotein Res. Group, Univ. Alberta, Edmonton, AB, T6G 2S2, Can.). Journal of Lipid Research, 37(3), 599-605 (English) 1996. CODEN: JLPRAW. ISSN: 0022-2275. Publisher: Lipid Research, Inc..

AB The rabbit cholesteryl ester transfer protein (CETP) was expressed in the methylotrophic yeast Pichia pastoris by introducing the CETP cDNA under the control of the methanol-inducible alc. oxidase promoter. The cDNA was cloned from in vitro amplified cDNA of rabbit liver mRNA. The nucleotide sequence of the cloned cDNA differed slightly from the previously published sequence that changed the amino acid sequence in six residues. Interestingly, five of these replacements are identical to the corresponding residues in human CETP. In addition, the encoded mature N-terminal sequence was changed from Cys-to Arg-Glu-Phe- to link the CETP sequence to the yeast acid phosphatase signal peptide. The culture medium of the transformed cells induced with 1% methanol contained both cholesteryl ester and triglyceride transfer activity comparable to that of rabbit plasma. Like rabbit plasma, the lipid transfer activity in the medium could be inhibited by monoclonal antibodies that block CE/TG transfer or TG transfer alone. Immunoblot anal. of the medium detected a major immunoreactive species of Mr = 80 K and minor species of Mr = 60-100 K. In spite of these differences, the specific transfer activity of the recombinant CETP was indistinguishable from that of rabbit plasma CETP of Mr = 74 K. N-Glycosidase F treatment converted both the recombinant and plasma CETP to a single species of Mr = 55 K. Both the plasma and recombinant CETP lost their activity after removal of N-linked carbohydrate and sialic acid. A single 55 K component was found in the cell-lysates. The intracellular form of the recombinant CETP was not modified by N-glycosidase F treatment. In conclusion, the recombinant CETP is synthesized as an inactive polypeptide that is processed and secreted as a functional glycoprotein. In addition, the N-terminal Cys residue of the plasma CETP is not required for its activity.

L8 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2006 ACS on STN

1992:529969 Document No. 117:129969 Synthesis and secretion of human lysozyme by methylotrophic yeasts. Davis, Geneva Ruth; Stillman, Cathy Ann; Brierley, Russell Arthur; Thill, Gregory Patrick; Vedvick, Thomas Scott (Salk Institute Biotechnology/Industrial Associates, Inc., USA). PCT Int. Appl. WO 9204441 A1 19920319, 90 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US6326 19910904. PRIORITY: US 1990-577994 19900905.

AB Expression cassettes for the efficient secretory manufacture of human lysozyme in methylotrophic yeasts are described. The gene is placed under control of a strong methanol-inducible promoter, and secretion is directed by the signal peptide of the Saccharomyces cerevisiae α-mating factor. The gene is stabilized by insertion into the genome. A series of vectors in which one or two copies of the human lysozyme gene were expressed from the Pichia pastoris AOX1 gene promoter using either the human or the S. cerevisiae signal peptides were constructed. These vectors were designed to integrate into the P. pastoris HIS4 gene. When the human signal sequence was used the yield of enzyme in the medium after 94 h growth on MeOH as sole C source was 16 mg lysozyme/L (RIA). When the S. cerevisiae signal sequence was used the yield was 155 mg/L and when two such expression cassettes were used this rose to 550 mg/L. Methods for purification of the enzyme from the culture medium are described.

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=> S GENE
      1068592 GENE
       406335 GENES
L9    1133217 GENE
                (GENE OR GENES)

=> S L1 (5A) L9
L10       1037 L1 (5A) L9

=> S (L1(5A)L2)(5A)L3
L11          0 (L1(5A)L2)(5A)L3
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=> S L4 (5A)L3
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L4 (5A)L3'
L12 73 L4 (5A)L3

=> S L2(5A)L3;S L1(5A)L3
L13 2 L2(5A)L3

L14 28 L1(5A)L3

=> S L13,L14
L15 30 (L13 OR L14)

=> S L15 NOT L8
L16 29 L15 NOT L8

=> D 1-29 CBIB ABS

L16 ANSWER 1 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2006:927180 Expression and secretion of natural N-terminal rBPTI with PHO I signal peptide in Pichia pastoris. Yang, Lili; He, Jinchao; Ma, Jie; Fan, Weiquan; Wang, Jianqiu; Wei, Chuanyu; Yan, Haowei; Yan, Weiqun (Institute of Frontier Medical Sciences, Jilin University, Changchun, Jilin Province, 130021, Peop. Rep. China). Jilin Daxue Xuebao, Yixueban, 31(6), 830-832 (Chinese) 2005. CODEN: JDXYA3. ISSN: 1671-587X. Publisher: Jilin Daxue Xuebao, Yixueban Bianjibu.
AB Objective: To express and secrete natural N-terminal recombinant bovine pancreatic trypsin inhibitor (rBPTI) with acid phosphatase (PHO I) signal peptide in Pichia pastoris. Methods: PHO I/bpti genes were inserted into the eukaryotic expression plasmid. The recombinant plasmid was transformed into Pichia pastoris (X-33) via electroporation. Results: The expression plasmid was constructed to contain correct sequence for PHO I/bpti genes. The rBPTI was expressed and secreted in X-33. An activity strain was selected with trypsin inhibitor experiment The expression supernatant was purified with cation exchange chromatog. to single peak, and SDS-PAGE indicated that the relative mol. mass was 6500. Conclusion: RBPTI with natural N-terminal sequence is successfully expressed in Pichia pastoris with signal peptide of PHO I.

L16 ANSWER 2 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2006:738044 Cloning and expression in Pichia pastoris of an alkaline mannanase gene. Tan, Xiuhua; Wu, Yuyong; Ma, Lixin; Jiang, Sijing (Laboratory of Molecular Microbiology + Gene Engineering, College of Life Science, Hubei University, Wuhan, 430062, Peop. Rep. China). Weishengwu Xuebao, 45(4), 543-546 (Chinese) 2005. CODEN: WSHPA8. ISSN: 0001-6209. Publisher: Kexue Chubanshe.
AB A strain containing alkaline mannanase gene was isolated from soil by functional plates and the genome library was constructed. From it, a mannanase gene TM1 was acquired and sequenced. The BLAST anal. showed a lower than 60% similarity of the amino acid sequence to those in GenBank and proved TM1 to be a new mannanase gene (GenBank accession number AY623,903). The new gene without signal peptide was cloned into the Pichia pastoris expression vector pHBM905C. The recombinant plasmid pHBM1,201 was digested by Sal I and transformed into Pichia pastoris KM71, GS115, SMD1,168, resp. All of the recombinant Pichia pastoris strains containing pHBM1,201 secreted functional β-mannanase. Because of its high mass of expression, the recombinant Pichia pastoris SMD1,168-3 containing pHBM1,201 was induced at shake flasks. The optimal temperature and pH of the β-mannanase produced by the recombinant strains were 55° and 7.5, resp. The enzymic activity for konjak powder reached 41.8 with a half life of 1 h. After keeping at 80° for 5 min, the enzymic activity declined from 77% to 11% and the enzymic activity could recover up to more than 60% when the temperature descended to 55°.

L16 ANSWER 3 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2006:678653 Document No. 145:138651 Expression of potato proteinase

inhibitor II in microbial hosts. Louie, Michael Tai-Man; Salamone, Peter (USA). U.S. Pat. Appl. Publ. US 2006154340 A1 20060713, 28 pp. (English). CODEN: USXXCO. APPLICATION: US 2005-31895 20050107.

AB The invention relates generally to a method for expressing potato proteinase inhibitor II (PI2) in a microbial host and, more specifically, to a method for producing com. quantities of PI2 using transformed strains of Pichia pastoris and Escherichia coli. Heterologous expression of PI2 in microbial hosts (Escherichia coli and Pichia pastoris) described in this invention overcomes the limitations of the extraction process. For expression in Escherichia coli, the PCR-amplified PI2 gene was cloned into the com. available expression plasmid pET32a (from Novagen) as a C-terminal fusion protein of thioredoxin (TrxA), resulting in plasmid pKBEPI-5. Plasmid pKBEPI-5 was then transformed into E. coli BL21 trxB(DE3) for the cytoplasmic production of a TrxA-PI2 fusion protein, after the cells were induced by 1 mM of IPTG. The advantage of this E. coli expression system is that milligram quantities of PI2 are produced in 1 L of E. coli cells under a fairly short period of time (1 day) since E. coli is a fast-growing organism. The essential elements of this invention that result in high-level expression of PI2 in E. coli include: (1) the powerful T7 promoter on plasmid pET32a expresses the trxA-PI2 gene at a high level; (2) the trxA gene of pET32a allows expression of PI2 as a TrxA-PI2 fusion protein. It is believed that the TrxA portion helps PI2 to fold properly and to remain soluble inside E. coli cells. The heat stability of TrxA also allows using simple heating as a purification step to precipitate most of the native E. coli proteins, leaving mostly the TrxA-PI2 fusion protein; (3) the internal His-tag between TrxA and PI2 allows further purification of the fusion protein; (4) the internal enterokinase site allows efficient removal of the TrxA portion from PI2 portion of the fusion protein. For expression in Pichia pastoris, the PCR-amplified PI2 gene was cloned as a C-terminal fusion with the Sacchromyces cerevisiae mating factor alpha prepro signal peptide (MFα) in the Pichia pastoris expression plasmid pKBPPI-3. The advantage of the PGAP-based P. pastoris expression system is that several hundred milligrams of PI2 can be produced in 1 L of P. pastoris cells. Multiple copies of the PI2-expression cassettes present in one recombinant host significantly contributed to the high expression level. The essential elements of this invention that result in high-level expression of PI2 in P. pastoris include: (1) the strong and constitutive PGAP promoter that drives the expression of the MFα-PI2 gene fusion. The PGAP promoter also allows the expression system to be developed into a full-scale production process; (2) the MFα secretion signal efficiently directs secretion of PI2 into the culture medium; (3) multiple copies of the PI2 expression cassette (or PI2 gene) in one P. pastoris strain significantly increases PI2 expression levels when compared to a strain with only 1 copy of the expression cassette.

L16 ANSWER 4 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2006:610030 Efficient expression and secretion of two co-produced xylanases from Aspergillus niger in Pichia pastoris directed by their native signal peptides and the Saccharomyces cerevisiae α-mating factor. Korona, Boguslawa; Korona, Dagmara; Bielecki, Stanislaw (Institute of Technical Biochemistry, Technical University of Lodz, Lodz, 90-924, Pol.). Enzyme and Microbial Technology, 39(4), 683-689 (English) 2006. CODEN: EMTED2. ISSN: 0141-0229. Publisher: Elsevier B.V..

AB The two genes encoding precursors of co-produced endo-1,4-β-D- xylanases, Xyn6 and XynB, were isolated from Aspergillus niger IBT-90 by using RT-PCR technique and expressed in Pichia pastoris under the control of the alc. oxidase I promoter. The secretion was driven by the Saccharomyces cerevisiae α-mating factor fused to mature xylanases and by the native 27-amino acid leader peptide of Xyn6 or 37-amino acid signal of XynB when the entire open reading frames of proteins were cloned. The secretion level of XynB directed by α-factor estimated at 140 mg l-1 was comparable to 150 mg l-1 with its own leader peptide; whereas in case of Xyn6, the yield was up to 180 and 220 mg l-1, resp. The N-termini of active recombinant Xyn6 and XynB indicated that their endogenous pre(pro)signals are effectively recognized and correctly processed in P. pastoris, like α-factor. These findings should contribute to develop the possibilities of application of the alternative secretion signals in P. pastoris. The initial studies revealed the different pH optima of Xyn6 (3.5) and XynB (5.0). The further anal. of individual gene products should enable to clarify the role of a particular enzyme in a complex xylanase system of A. niger.

L16 ANSWER 5 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2005:1179915 Document No. 144:167197 Signal peptide peptidase promotes the formation of hepatitis C virus non-enveloped particles and is captured on the viral membrane during assembly. Majeau, Nathalie; Gagne, Valerie; Bolduc, Marilene; Leclerc, Denis (Infectious Disease Research Centre, University of Laval, Quebec, QC, G1V 4G2, Can.). Journal of General Virology, 86(11), 3055-3064 (English) 2005. CODEN: JGVIAY. ISSN: 0022-1317. Publisher: Society for General Microbiology.

AB The maturation of the core protein (C) of Hepatitis C virus (HCV) is controlled by the signal peptidase (sp) and signal peptide peptidase (spp) of the host. To date, it remains unknown whether spp cleavage influences viral infectivity and/or the assembly process. Here, evidence is provided that cleavage by spp is not required for assembly of nucleocapsid-like particles (NLPs) in yeast (Pichia pastoris). The immature NLPs (not processed by spp) show a d. of 1·11 g mL-1 on sucrose gradients and a diameter of 50 nm. Co-expression of human spp (hspp) with C generates the 21 kDa mature form of the protein and promotes the accumulation of non-enveloped particles. The amount of non-enveloped particles accumulating in the cell was correlated directly with the expression level of hspp. Furthermore, immunocapture studies showed that hspp was embedded in the membranes of enveloped particles. These results suggest that maturation of the C protein can occur after formation of the enveloped particles and that the abundance of hspp influences the types of particle accumulating in the cells.

L16 ANSWER 6 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2005:508614 Document No. 143:192373 Functional Expression in Pichia pastoris of an Acidic Pectin Methylesterase from Jelly Fig (Ficus awkeotsang). Peng, Chi-Chung; Hsiao, Eric S. L.; Ding, Joe L. C.; Tzen, Jason T. C. (Graduate Institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan). Journal of Agricultural and Food Chemistry, 53(14), 5612-5616 (English) 2005. CODEN: JAFCAU. ISSN: 0021-8561. Publisher: American Chemical Society.

AB A cDNA fragment encoding an acidic pectin methylesterase (PME) of jelly fig achene was successfully expressed in Pichia pastoris under the control of the glyceraldehydes-3-phosphate dehydrogenase promoter. The recombinant PME was produced as a secretory protein by N-terminal fusion of a cleavable prepropeptide for signal trafficking, and thus easily harvested from the culture medium. Compared with native N-glycosylated PME (38 kDa) purified from jelly fig achenes, this recombinant PME (45 kDa) appeared to be hyperglycosylated. Activity staining indicated that the recombinant PME was functionally active. Yet the hyperglycosylated recombinant PME possessed thermostability and enzymic capability over a broad pH range equivalent to those of the native PME. The success of functional production of this acidic jelly fig PME in P. Pastoris has significantly broadened its applications in industry.

L16 ANSWER 7 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2005:330125 Document No. 143:242743 Cloning and heterologous expression of the antibiotic peptide (ABP) genes from Rhizopus oligosporus NBRC 8631. Yamada, Osamu; Sakamoto, Kazutoshi; Tominaga, Mihoko; Nakayama, Tasuku; Koseki, Takuya; Fujita, Akiko; Akita, Osamu (National Research Institute of Brewing, Higashi-Hiroshima, 739-0046, Japan). Bioscience, Biotechnology, and Biochemistry, 69(3), 477-482 (English) 2005. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB We carried out protein sequencing of purified Antibiotic Peptide (ABP), and cloned two genes encoding this peptide as abp1 and abp2, from Rhizopus oligosporus NBRC 8631. Both genes contain an almost identical 231-bp segment, with only 3 nucleotide substitutions, encoding a 77 amino acid peptide. The abp gene product comprises a 28 amino acid signal sequence and a 49 amino acid mature peptide. Northern blot anal. showed that at least one of the abp genes is transcribed in R. oligosporus NBRC 8631. A truncated form of abp1 encoding only the mature peptide was fused with the α-factor signal peptide and engineered for expression in Pichia pastoris SMD1168H. Culture broth of the recombinant Pichia displayed ABP activity against Bacillus subtilis NBRC 3335 after induction of heterologous gene expression. This result indicates that

mature ABP formed the active structure without the aid of other factors from R. oligosporus, and was secreted.

L16 ANSWER 8 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2005:117432 Document No. 142:409764 Engineering of a Pichia pastoris expression system for secretion of high amounts of intact human parathyroid hormone. Vad, Randi; Nafstad, Eidi; Dahl, Linn Anita; Gabrielsen, Odd S. (Department of Molecular Biosciences, University of Oslo, Oslo, N-0316, Norway). Journal of Biotechnology, 116(3), 251-260 (English) 2005. CODEN: JBITD4. ISSN: 0168-1656. Publisher: Elsevier B.V..

AB Human parathyroid hormone (hPTH) is involved in calcium metabolism, and the unique ability of this hormone to stimulate bone growth makes it a promising agent in the treatment of osteoporosis. We have engineered the methylotrophic yeast Pichia pastoris for the production of over 300 mg intact hPTH per L growth medium. The presence of 10 mM EDTA in the culture medium was essential to obtain this high hormone yield, indicating that metallopeptidases are mainly responsible for the otherwise instability of hPTH. Furthermore, the secretion process of hPTH was considerably improved by coexpression of Saccharomyces cerevisiae protein disulfide isomerase (ScPDI). Since hPTH does not contain any cysteine residues, this effect may be indirect or ascribed to the chaperone activity of PDI. Contrary to the situation in S. cerevisiae, use of a protease-deficient host strain provided no addnl. advantage. The hormone secreted by P. pastoris was not subjected to proteolytic processing by Kex2p in the two internal tribasic sites, nor were any C-terminal truncated hPTH forms detected. However, the P. pastoris hPTH producing transformants cosecreted ubiquitin to the culture medium, possibly as a result of a stress-related response.

L16 ANSWER 9 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2004:693717 Document No. 141:259337 Signal peptide of eosinophil cationic protein is toxic to cells lacking signal peptide peptidase. Wu, Chia-Mao; Chang, Margaret Dah-Tsyr (Department of Life Science, Institute of Molecular and Cellular Biology, National Tsing Hua University, Taichung, 30013, Taiwan). Biochemical and Biophysical Research Communications, 322(2), 585-592 (English) 2004. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Elsevier.

AB Eosinophil cationic protein (ECP) is a toxin secreted by activated human eosinophils. The properties of mature ECP have been well studied but those of the signal peptide of ECP (ECPsp) are not clear. In this study, several chimeric proteins containing N-terminal fusion of ECPsp were generated, and introduced into Escherichia coli, Pichia pastoris, and human epidermoid carcinoma cell line A431 to study the function of ECPsp. The authors found that expression of ECPsp chimeric proteins inhibited the growth of E. coli and P. pastoris but not A431 cells. Primary sequence anal. and in vitro transcription/translation of ECPsp have revealed that it is a potential substrate for human signal peptide peptidase (hSPP), an intramembrane protease located in endoplasmic reticulum. In addition, knockdown of the hSPP mRNA expression in ECPsp-eGFP/A431 cells caused the growth inhibitory effect, whereas complementally expression of hSPP in P. pastoris system rescued the cell growth. Taken together, the authors have demonstrated that ECPsp is a toxic signal peptide, and expression of hSPP protects the cells from growth inhibition.

L16 ANSWER 10 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2004:316848 Document No. 141:2012 Expression of porcine interferon-γ gene in Pichia pastoris and its effect of inhibiting porcine reproductive and respiratory syndrome virus. Wan, Jianqing; Wu, Wenxue; Xia, Chun (College of Veterinary Medicine, Chinese Agricultural University, Beijing, 100094, Peop. Rep. China). Shengwu Gongcheng Xuebao, 18(6), 683-686 (Chinese) 2002. CODEN: SGXUED. ISSN: 1000-3061. Publisher: Kexue Chubanshe.

AB The recombinant porcine interferon-γ (rPoIFN-γ) cDNA lacking the signal peptide was expressed in Pichia pastoris GS115 strain, and the effect of rPoIFN-γ on porcine reproductive and respiratory syndrome virus (PRRSV) was studied. The PoIFN-γ gene was inserted into integrative vector pHIL-S1, and the recombinant GS115 strain (pHIL-S1/PoIFN-γ) was constructed by homolog recombinant. The rPoIFN-γ protein was 18 kD with

an expressing yield of 18% assayed by SDS-PAGE and Western blot, resp. The antiviral activity of rPoIFN-γ was 450-540 u mL-1. In addition, the anti-PRRSV effect of rPoIFN-γ was determined using CPE50 method. The high concentration of rPoIFN-γ could inhibit PRRSV on Marc-145 cell line. The rPoIFN-γ was a potential drug for prevention and treatment of various kinds of viral pig diseases.

L16 ANSWER 11 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2004:52539 Document No. 140:123660 Recombinant fibrinogen production in Pichia yeast. Tojo, Naoko; Miyagi, Ikuko; Miura, Masami; Oi, Hideyuki (Mitsubishi Welpharma Co., Japan). Jpn. Kokai Tokkyo Koho JP 2004016055 A2 20040122, 22 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2002-173520 20020614.

AB A method for producing recombinant fibrinogen having complete mol. structure (Aα-Bβ-γ)2 in Pichia yeast by genetic engineering, is disclosed. The method comprises (1) providing a plasmid containing a promoter capable of expressing heterologous protein in Pichia yeast, signal sequence, and fibrinogen Aα chain, Bβ chain and γ chain-coding sequence; (2) transforming Pichia yeast with the plasmid; (3) culturing the stably transformed yeast under the condition of pH 5-8 to produce fibrinogen; and (4) purifying produced fibrinogen. A protease deficient strain of Pichia yeast or serine proteinase inhibitor is used. Cation exchange or gel filtration is used for purification Production of recombinant human fibrinogen in Pichia pastoris using GAP (glyceraldehyde-3-phosphate dehydrogenase) or alc. oxidase (AOX2) promoter, is described. Addition of proteinase inhibitors PMSF, aprotinin, and chymostatin completely blocked the fibrinogen degradation The recombinant fibrinogen was purified by gel filtration chromatog. Other conditions for optimizing the production, pH, YP (yeast extract and peptone) concentration, carbon source, etc., were explored.

L16 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2003:860564 Document No. 140:247808 Secretory expression of human osteoprotegerin in Pichia pastoris and bioactivity analysis of the recombinant protein. Liu, Ji-zhong; Chin, Su-min; Li, Yi; Hu, Yun-yu; Ji, Zong-ling; Yang, Tong-tao (Dep. Biochemistry Molecular Biology, Fourth Military Med. Univ., Xi'an, 710032, Peop. Rep. China). Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao, 19(5), 566-571 (English) 2003. CODEN: ZSHXF2. ISSN: 1007-7626. Publisher: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui.

AB To express human osteoprotegerin (OPG) in Pichia pastoris and determine its inhibitive effects on osteoclast differentiation and bone resorptive function in vitro. Synthetic oligonucleotides were used to amplify human osteoprotegerin gene by RT-PCR from total RNA of human osteosarcoma cell line MG63. The OPG cDNA coding for 410 amino acid residues with native signal peptide was inserted into Pichia pastoris expression vector pPICZ-B containing AOX1 promoter and 18-nucleotide fragment encoding hexahistidine residues (6 + His) at the 3' end, a recombinant expression plasmid pPICZB-OPG was constructed and transformed to yeast host strain GS115, and human OPG was expressed under the induction of 3% methanol for 5 days. SDS-PAGE and Western blot showed that the expressed product existed in supernatant in the form of soluble mol., and mol. weight of recombinant protein was about 66 kD and it could react specifically with anti-OPG antibody. The addition of purified OPG-6His protein (100 ng/mL) could decrease the number of dentin resorption pits and tartrate-resistant acid phosphatase (TRAP)-pos. multinucleated cells in vitro ($P < 0.05$). A highly efficient recombinant expression system was developed which produced a native and functional form of human OPG in the Pichia pastoris.

L16 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2003:444821 Document No. 139:229304 Influence of signal peptide sequences on the expression of heterogeneous proteins in Pichia pastoris. Xiong, Aisheng; Peng, Rihe; Li, Xian; Fan, Huiqin; Yao, Quanhong; Guo, Meijin; Zhang, Siliang (Shanghai Key Laboratory of Agricultural Genetics and Breeding, Agro-Biotechnology Research Center, Shanghai Academy of Agricultural Sciences, Shanghai, 201106, Peop. Rep. China). Shengwu Huaxue Yu Shengwu Wuli Xuebao, 35(2), 154-160 (Chinese) 2003. CODEN: SHWPAU. ISSN: 0582-9879. Publisher: Shanghai Kexue Jishu Chubanshe.

AB Pichia pastoris has been developed to be a very efficient expression host for the heterogeneous proteins since its alc. promoter was isolated and cloned, and its transformation technique was established. The signal peptide sequences and its effect on the secretion expression of heterogeneous proteins in Pichia pastoris were studied. The Saccharomyces cerevisiae mating factor α prepro-leader sequence was synthesized using successive PCR and designated as MF4I, then ten different signal sequences were constructed by adding the N-terminal residues of Pichia pastoris alc. oxidase 1 (Aox1) protein to the N-terminal of the MF4I and used for directing phytase gene secretion in Pichia pastoris. The secretion of phytase was increased in Pichia pastoris strains containing new signal sequence. Among these strains, the phytase secretion was the highest in strain containing signal sequence added with A, I, P three Aox1 N-terminal residues; and the phytase secretion of Pichia pastoris was 90 mg L-1 in flake. The secretion was six-fold than that with original Saccharomyces cerevisiae mating factor α prepro-leader sequence. In addition, insert of ten residues E E A E A E A E P K can further increase the phytase secretion by 35%, and the secretion reached 120 mg L-1.

L16 ANSWER 14 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2003:407363 Document No. 139:392749 Production and characterization of the Talaromyces stipitatus feruloyl esterase FAEC in Pichia pastoris: identification of the nucleophilic serine. Crepin, Valerie F.; Faulds, Craig B.; Connerton, Ian F. (School of Biosciences, Division of Food Science, University of Nottingham, Loughborough, LE12 5RD, UK). Protein Expression and Purification, 29(2), 176-184 (English) 2003. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB Feruloyl esterases constitute an interesting group of enzymes that have the potential for use over a broad range of applications in the agri-food industries. We report the over-expression and characterization of a novel feruloyl esterase exhibiting broad substrate specificity from Talaromyces stipitatus (FAEC) in Pichia pastoris. Using various gene constructions, we have investigated the use of alternative signal peptides to produce an authentic feruloyl esterase featuring the N-terminal sequence determined for the native enzyme. We demonstrate that addnl. amino acids at the N-terminus of the FAEC sequence do not influence the catalytic capacity of the enzyme, and that the nature of the signal sequence has a limited effect on the yield of the secreted enzyme, with the T. stipitatus FAEC signal sequence producing 297 mg L-1, the Neurospora crassa Fae-1 260 mg L-1, and the Saccharomyces cerevisiae α-factor secretion signal 214 mg L-1. Mature FAEC contains two internal peptide sequences that correspond with the consensus motif G-X-S-X-G that contains the catalytic serine nucleophile, which is conserved in the esterase enzyme superfamily. The serine residues at the center of these peptide motifs have been independently mutated and the corresponding enzymes have been over-expressed in P. pastoris to identify the candidate nucleophilic residue responsible for catalyzing the enzymic reaction. Purified recombinant FAEC containing S465A retained the esterase activity and appeared unaffected by the amino acid modification. In contrast, FAEC activity containing S166A was below the HPLC detection limit, suggesting that serine 166 constitutes the nucleophile.

L16 ANSWER 15 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2003:360771 Document No. 138:380398 Secretion of active-form Streptoverticillium mobaraense transglutaminase by methanol-metabolizing yeast: Processing of the pro-transglutaminase by a co-secreted protease. Sakai, Yasunori; Matsui, Hiroshi; Kato, Nobuo (Ajinomoto Co., Inc., Japan). Jpn. Kokai Tokkyo Koho JP 2003135058 A2 20030513, 47 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2002-56323 20020301. PRIORITY: JP 2001-249771 20010821.

AB Biosynthetic production of Actinomyces transglutaminase in methanol-metabolizing yeast is disclosed. Transglutaminase coding sequence is attached to a yeast derived signal peptide coding sequence, and methanol-metabolizing yeast is transformed. A modified pro-transglutaminase having a protease cleavage site inserted between pro and mature protein sequence, and having mutations in its glycosylation site, is expressed and secreted in host yeast, and processed with a protease, to yield a mature transglutaminase. Production of Streptoverticillium mobaraense transglutaminase in Candida boidinii is described. Pro- or mature transglutaminase having a yeast α factor signal peptide and Kex2 endopeptidase cleavage site were expressed. Transglutaminase mutants having Asparagine (N29, N327, N342) substituted with Glutamine, were also produced.

L16 ANSWER 16 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2002:932925 Document No. 138:218384 Characterization of a chemosensory protein (ASP3c) from honeybee (Apis mellifera L.) as a brood pheromone carrier. Briand, Loic; Swasdipan, Nicharat; Nespoulous, Claude; Bezirard, Valerie; Blon, Florence; Huet, Jean-Claude; Ebert, Paul; Pernollet, Jean-Claude (Biochimie et Structure des Proteines, Unite de recherches INRA 477, Jouy-en-Josas, F-78352, Fr.). European Journal of Biochemistry, 269(18), 4586-4596 (English) 2002. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Blackwell Science Ltd..

AB We report the cloning of a honeybee chemosensory proteins (CSP) gene called ASP3c, as well as the structural and functional characterization of the encoded protein. The protein was heterologously secreted by the yeast Pichia pastoris using the native signal peptide . ASP3c disulfide bonds were assigned after trypsinolysis followed by chromatog. and mass spectrometry combined with microsequencing. The pairing (Cys(I)-Cys(II), Cys(III)-Cys(IV)) was found to be identical to that of Schistocerca gregaria CSPs, suggesting that this pattern occurs commonly throughout the insect CSPs. CD measurements revealed that ASP3c mainly consists of α-helixes, like other insect CSPs. Gel filtration anal. showed that ASP3c is monomeric at neutral pH. Using ASA, a fluorescent fatty acid anthroyloxy analog as a probe, ASP3c was shown to bind specifically to large fatty acids and ester derivs., which are brood pheromone components, in the micromolar range. It was unable to bind tested general odorants and other tested pheromones (sexual and nonsexual). This is the 1st report on a natural pheromonal ligand bound by a recombinant CSP with a measured affinity constant

L16 ANSWER 17 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2002:884448 Document No. 138:88709 Effect of different signal peptides on the secretion of hTFF3 in Pichia pastoris. Wang, Yanru; An, Lin; Li, Lingyuan; Ru, Binggen (National Laboratory of Protein Engineering, College of Life Science, Peking University, Beijing, 100871, Peop. Rep. China). Yaowu Shengwu Jishu, 9(2), 74-78 (Chinese) 2002. CODEN: YSJIFO. ISSN: 1005-8915. Publisher: Yaowu Shengwu Jishu Bianjibu.

AB The effect of different signal peptides on the expression of the native N-terminus human trefoil factor 3 (hTFF3) in Pichia pastoris was studied. Two kinds of Pichia pastoris expression vectors were constructed, one contained the STE 13 signal sequence and another without the sequence. The two vectors were transformed into the Pichia pastoris. Mol. weight of the expressed peptides was about 13 kD identified by SDS-PAGE and Western-blotting. N-terminus amino acids anal. showed one yeast expressed TFF3 with the native N-terminus and the right mol. weight, while another recombinant protein had a hybrid leader sequence GluAla at the N-terminus and had two protein MS peaks.

L16 ANSWER 18 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2002:727807 Document No. 138:1066 Yeast Pichia pastoris strain as a producer of human fibroblast interferon, recombinant plasmid DNA pHIF and a method of its construction. Padkina, M. V.; Parfenova, L. V.; Sambuk, E. V.; Smirnov, M. N. (Obshchestvo s Ogranichennoi Otvetstvennost'yu "Biotekh", Russia). Russ. RU 2180687 C1 20020320, No pp. given (Russian). CODEN: RUXXE7. APPLICATION: RU 2000-118716 20000705.

AB FIELD: biotechnol., genetic engineering, mol. biol., microbiol. and medicinal industry. SUBSTANCE: recombinant plasmid DNA pHIF has fragment EcoRI - XhoI of bacterial-yeast vector pPIC9 and fragment XhoI - EcoRI including encoding moiety of human gene β-interferon with exception the region encoding signal peptide. Gene encoding beta-interferon is prepared by polymerase chain reaction method and using pHBI template with help of direct and inverse primer. Plasmid pHIF is transformed into the yeast strain to obtain the transformed strain Pichia pastoris PS99 (pHIF). Invention ensures to provide preparing human β-interferon accumulating in cultural fluid. Invention can be used for preparing human fibroblast interferon. EFFECT: improved method of interferon production

L16 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2002:611179 Document No. 137:368663 Expression and purification of a small cytokine growth-blocking peptide from armyworm Pseudaletia separata by an

optimized fermentation method using the methylotrophic yeast Pichia pastoris. Koganesawa, Nozomi; Aizawa, Tomoyasu; Shimojo, Hiroshi; Miura, Kazunori; Ohnishi, Atsushi; Demura, Makoto; Hayakawa, Yoichi; Nitta, Katsutoshi; Kawano, Keiichi (Graduate School of Science, Division of Biological Sciences, Hokkaido University, Sapporo, 060-0810, Japan). Protein Expression and Purification, 25(3), 416-425 (English) 2002. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB A small multifunctional cytokine, growth-blocking peptide (GBP), from the armyworm Pseudaletia separata larvae was expressed as a soluble and active recombinant peptide in the methylotrophic yeast Pichia pastoris. An expression vector for GBP secretion was constructed using vector pPIC9, and GBP was expressed under the control of the alc. oxidase (AOX1) promoter. Although we first tried to cultivate GBP in shake flask cultures, the yield was low, probably due to proteolysis of the recombinant protein. To overcome this problem, we utilized a high-d. fermentation method. The pH of the medium in the fermenter was kept at 3.0, and the medium was collected within 48 h post methanol shift to minimize exposure of the target peptide to proteases. Recombinant GBP was purified through three reverse-phase HPLC columns. We characterized the 25 amino acid GBP by mol. mass spectrometry and amino acid sequencing. Plasmatocyte spreading, one of the activities of GBP, was similar between chemical synthesized GBP and purified recombinant GBP. Up to 50 mg GBP was recovered per 1 L of yeast culture supernatant.

L16 ANSWER 20 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2002:533549 Document No. 138:101562 Glutamic acid and alanine spacer is not necessary for removal of MFα-1 signal sequence fused to the human growth hormone produced from Pichia pastoris. Eurwilaichitr, Lily; Roytrakul, Sittiruk; Suprasongsin, Chittiwat; Manitchotpisit, Pennapa; Panyim, Sakol (Institute of Molecular Biology and Genetics, BIOTEC Training Center for Genetic Engineering and Biotechnology, Mahidol University, Nakhonpathom, 73170, Thailand). World Journal of Microbiology & Biotechnology, 18(6), 493-498 (English) 2002. CODEN: WJMBEY. ISSN: 0959-3993. Publisher: Kluwer Academic Publishers.

AB Human growth hormone (hGH) cDNA was synthesized using codons preferred by Escherichia coli, except for the first 20 amino acids, which were changed to that preferred by Saccharomyces cerevisiae and Pichia pastoris. Polymerase chain reaction (PCR) overlapping approach was employed to create synthetic hGH without glutamic acid-alanine (glu-ala), or with one and two glu-ala spacers (hGH, hGH1 and hGH2, resp.). The necessity of a glu-ala spacer in the cleavage of S. cerevisiae alpha mating factor-1 (MFα-1) secretion signal from the synthetic hGH was also investigated. Three types of hGH constructs were integrated into P. pastoris genome, the zeocin-resistant transformants were selected and expression of hGH was determined A 22-kDa band of secreted hGH was further determined by N-terminal peptide sequencing. The result suggested that the removal of glu-ala from the hGH1 and hGH2 was not efficient and only the hGH construct showed the complete cleavage of the signal sequence, giving a similar N-terminus as the mature hGH. HGH expression was optimized to increase the yield of the protein from the hGH construct (no glu-ala) to 190 mg/l from a 10-mL induction medium.

L16 ANSWER 21 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN

2001:812500 Document No. 136:84742 Comparison of three signals for secretory expression of recombinant human midkine in Pichia pastoris. Murasugi, Akira; Tohma-Aiba, Yumiko (Meiji Cell Technology Center, Meiji Milk Products Co., Ltd., Kanagawa, 250-0862, Japan). Bioscience, Biotechnology, and Biochemistry, 65(10), 2291-2293 (English) 2001. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB The secretion signals of Saccharomyces cerevisiae α mating factor, human midkine itself, and P. pastoris acid phosphatase, were tried for the expression of human midkine under the control of the AOX1 gene promoter in P. pastoris. Approx. 28 mg/L, 1.5 mg/L, and 0.2 mg/L of midkine were secreted by using the α mating factor pre-pro-sequence, the midkine signal sequence, and the phosphatase signal sequence in flask cultures, resp.

L16 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2001:713897 Document No. 135:283954 Pichia pastoria expression system using T7 RNA polymerase. Hua, Zichun; Wu, Xiaoyang (Nanjing Univ., Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1291652 A 20010418, 7 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2000-133288 20001201.

AB One Pichia (P.) pastoria expression system is described. The expression vector contains T7 RNA Polymerase gene regulated by or not by methanol, a desired gene which is under control of T7 promoter, and a marker gene for selection. Specifically, the expression cassette comprises T7 promoter, α factor signal peptide coding sequence, multiple cloning sites for desired gene insertion, and T7 transcription terminator.

L16 ANSWER 23 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2000:911446 Document No. 134:81767 Pichia methanolica glyceraldehyde 3-phosphate dehydrogenase 1 promoter and terminator and their use for expressing genes of interest in yeast. Miller, Brady G.; Sloan, James S.; Raymond, Christopher K.; Vanaja, Erica (Zymogenetics, Inc., USA). PCT Int. Appl. WO 2000078978 A1 20001228, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US16671 20000616. PRIORITY: US 1999-PV140703 19990624.

AB Transcription promoter and terminator sequences from the Pichia methanolica glyceraldehyde-3-phosphate dehydrogenase 1 gene (GAP1 gene) are disclosed. The sequences are useful within DNA constructs for the production of proteins of interest in cultured P. methanolica cells. Within the expression vectors, a GAP1 promoter and/or a GAP1 terminator is operably linked to A DNA segment encoding the protein of interest. The invention provides an exemplary vector pTAP76 which contains a function transcription promoter of GAP1 gene to drive the gene expression of a protein linked to Saccharomyces cerevisiae α-factor prepro sequence, a transcription terminator of a P. methanolica AUG1 or GAP1 gene, and P. methanolica ADE2 gene as the selection marker. Expression of heterologous genes from the GAP1 promoter is tested using LacZ and GFP (green fluorescent protein) reporter genes. A P. methanolica strain deficient for vacuolar proteases is generated for gene expression by disrupting PEP4 and PRB1 genes.

L16 ANSWER 24 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
2000:475684 Document No. 133:115895 Production of recombinant monellin using methylotrophic yeast expression system. Duan, Lingxun (Genway Biotech, Inc., USA). PCT Int. Appl. WO 2000040603 A2 20000713, 35 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US29213 19991209. PRIORITY: US 1998-PV114529 19981231.

AB The present invention relates to a single-chain monellin-like protein which is stable and which is at least 100-fold sweeter than sucrose on a weight basis. The present invention also relates to a nucleic acid encoding said monellin-like protein. Preferably, the nucleic acid further comprises a promoter and a signal sequence for directing expression and secretion of the encoded monellin-like protein in the methylothrophic yeast Pichia pastoris. The present invention further relates to a recombinant Pichia pastoris cell containing the nucleic acid encoding the monellin-like protein, a process for producing the monellin-like protein from the recombinant Pichia pastoris, and the product of the process. Thus, the plasmid pGWYS1 was constructed containing the P. pastoris glyceraldehyde 3-phosphate dehydrogenase promoter linked to the Saccharomyces cerevisiae α mating factor signal sequence fused to a single chain

monellin gene. The monellin gene contained the B chain linked to the A chain via a Gly linker. The single-chain monellin was produced by culturing P. pastoris transformed with pGWYS1.

L16 ANSWER 25 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
1999:656733 Document No. 132:20237 Functional phytohemagglutinin (PHA) and Galanthus nivalis agglutinin (GNA) expressed in Pichia pastoris correct N-terminal processing and secretion of heterologous proteins expressed using the PHA-E signal peptide. Raemaekers, Romaan J. M.; De Muro, Laura; Gatehouse, John A.; Fordham-Skelton, Anthony P. (Department of Biological Sciences, University of Durham, Durham, DH1 3LE, UK). European Journal of Biochemistry, 265(1), 394-403 (English) 1999. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Blackwell Science Ltd..

AB Phytohemagglutinin (Phaseolus vulgaris agglutinin; PHA; E- and L-forms) and snowdrop lectin (Galanthus nivalis agglutinin; GNA) were expressed in Pichia pastoris using native signal peptides, or the Saccharomyces α-factor preprosequence, to direct proteins into the secretory pathway. PHA and GNA were present as soluble, functional proteins in culture supernatants when expressed from constructs containing the α-factor preprosequence. The recombinant lectins, purified by affinity chromatog., agglutinated rabbit erythrocytes at concns. similar to the resp. native lectins. However, incomplete processing of the signal sequence resulted in PHA-E, PHA-L and GNA with heterogeneous N-termini, with the majority of the protein containing N-terminal extensions derived from the α-factor prosequence. Polypeptides in which most of the α-factor prosequence was present were also glycosylated. Inclusion of Glu-Ala repeats at the C-terminal end of the α-factor preprosequence led to efficient processing N-terminal to the Glu-Ala sequence, but inefficient removal of the repeats themselves, resulting in polypeptides with heterogeneous N-termini still containing N-terminal extensions. In contrast, PHA expressed with the native signal peptide was secreted, correctly processed, and also fully functional. No expression of GNA from a construct containing the native GNA signal peptide was observed The PHA-E signal peptide directed correct processing and secretion of both GNA and green fluorescent protein (GFP) when used in expression constructs, and is suggested to have general utility for synthesis of correctly processed proteins in Pichia.

L16 ANSWER 26 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
1999:38076 Document No. 130:205687 Production of recombinant human bile salt stimulated lipase and its variant in Pichia pastoris. Sahasrabudhe, Anjali V.; Solapure, Suresh M.; Khurana, Rajeev; Suryanarayan, Vepa; Ravishankar, Sudha; deSousa, Sunita M.; Das, Goutam (Astra Research Centre India, Bangalore, 560003, India). Protein Expression and Purification, 14(3), 425-433 (English) 1998. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic Press.

AB HBSSL and its truncated variant hBSSL-C cDNA clones were expressed in Pichia pastoris using two different signal peptides, native signal peptide and invertase signal peptide, resp., to facilitate secretion of the recombinant proteins into the culture medium. Both recombinant proteins were secreted into the culture medium to a level of 45-50 mg/L in shake flask cultures. Native signal peptide of hBSSL was recognized in P. pastoris and was cleaved at the same site as in humans. The level of expression of the hBSSL gene was found to be dependent on the number of its copies integrated into the host chromosome. The multicopy transformant clone was found to be very stable. When grown and induced in a fermentor, the level of accumulation of the recombinant hBSSL in the culture medium improved from 50 mg/L in shake flask cultures to 300 mg/L. The recombinant hBSSL purified from the culture supernatant was found to be similar to the native hBSSL in its biochem. properties except for the lectin-binding profile. (c) 1998 Academic Press.

L16 ANSWER 27 OF 29 CAPLUS COPYRIGHT 2006 ACS on STN
1999:21762 Document No. 130:138296 Recombinant preparation and secretion of Fab fragments of humanized anti-FcεRI receptors antibodies in Pichia pastoris. Takahashi, Kyoko; Yuki, Toshifumi; Takai, Toshiaki; Ra, Tomoyasu (Asahi Breweries, Ltd, Japan; Torii Yakuhin K. K.; Nikka Whisky Distilling Co., Ltd.). Jpn. Kokai Tokkyo Koho JP 11000174 A2 19990106 Heisei, 13 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1997-171232

19970613.

AB Disclosed is a method for the large-scale production of Fab fragments of humanized anti-FcεRI α chain by expression of the gene in Pichia pastoris, which gene contains the signal sequence of gene PHO1 from P. pastoris to replace the signal sequence of the antibody. Plasmid pAO815-PHO1-L,H; which consists of the AOX1 promoter of P. pastoris, the signal sequence of gene PHO1, and the gene encoding humanized anti-FcεRI α chain antibody of clone Hu.cRA2; was prepared and used for the transformation of P. pastoris. The Fab fragments purified from the culture medium of the transgenic P. pastoris were also characterized.

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1997:318212 Document No. 126:289025 Pichia acaciae killer toxin secretory leader comprising signal peptide with peptidase cleavage site for heterologous protein recombinant expression and secretion by host cell. Crawford, Kenneth; Zavor, Isabel; Bishop, Robert J.; Inns, Michael A. (Chiron Corporation, USA; Crawford, Kenneth; Zavor, Isabel; Bishop, Robert J.; Inns, Michael A.). PCT Int. Appl. WO 9712044 A2 19970403, 48 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US15329 19960925. PRIORITY: US 1995-4264 19950925; US 1995-4327 19950926.

AB A method to produce and secrete heterologous proteins that are free of addnl. N-terminal amino acids is presented using the Pichia acaciae killer toxin secretory leader. Expression vectors can be independently replicating or integrated into the host genome. Host cells can be eukaryote or prokaryote. Examples include insulin-like growth factor 1 gene expression using the pHIL-A1 vector in Pichia pastoris or SF9 host cells. The leader is a signal peptide plus peptidase cleavage site.

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1992:606374 Document No. 117:206374 Pichia pastoris acid phosphatase gene and its use for preparation of expression cassette. Buckholz, Richard G. (Phillips Petroleum Co., USA). Eur. Pat. Appl. EP 495208 A2 19920722, 39 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1991-121452 19911213. PRIORITY: US 1990-627539 19901214.

AB The gene for acid phosphatase (PHO1) Pichia pastoris is cloned and the regulatory regions and signal sequence of the gene used in the expression of foreign genes. A method for directing integration at the P. pastoris PHO1 locus utilizing a vector containing the 5' and 3' flanking sequences of P. pastoris PHO1 and a method for identifying the P. pastoris transformants having gene PHO1 disrupted due to integration are also described. The signal sequence of the PHO1 gene was used to promote secretion of invertase and tissue plasminogen activator in P. pastoris showed that it was as efficient or more efficient than the native signal sequence. Construction of gene PHO1-integrating vectors and yeast expression vectors containing the regulatory sequence of gene PHO1 was also shown.